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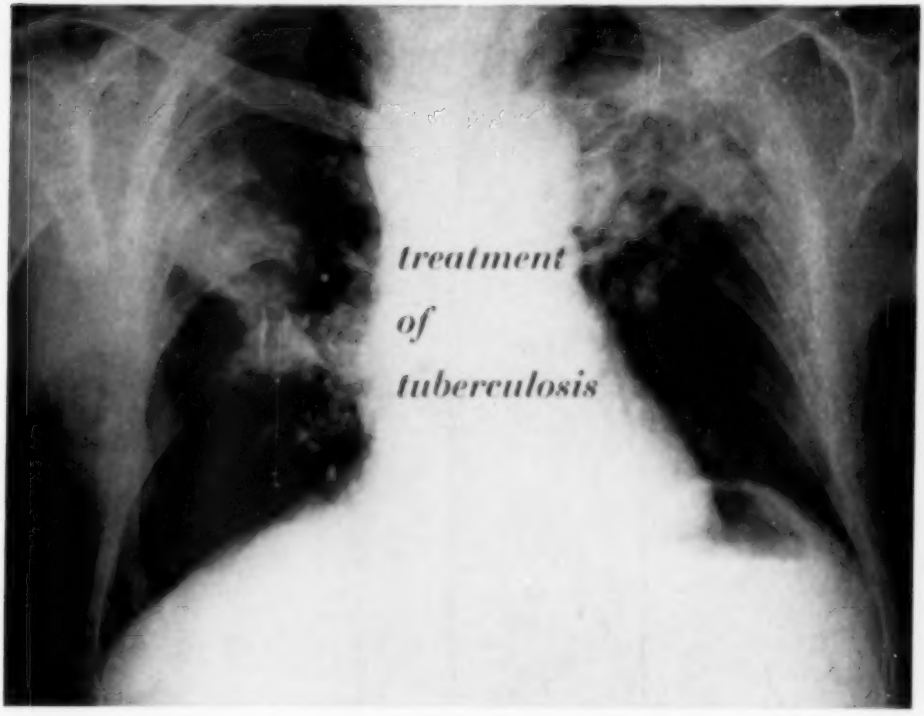

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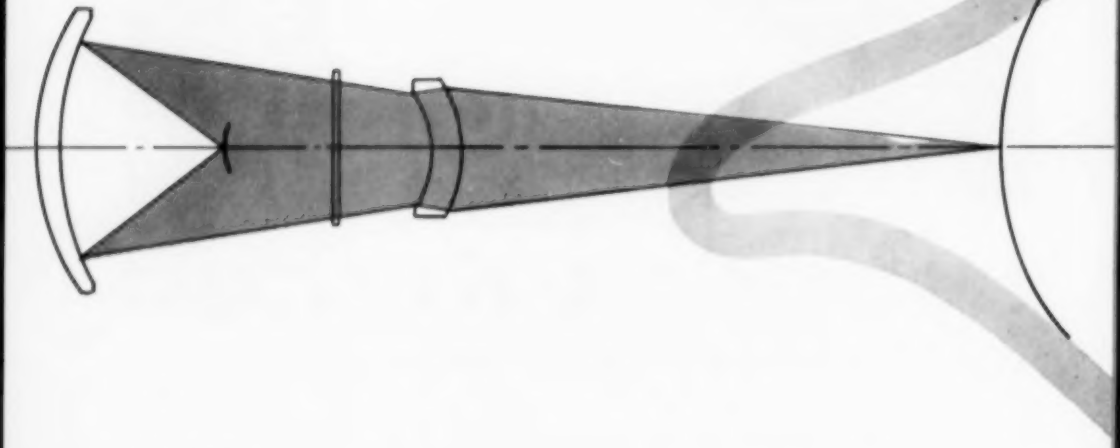

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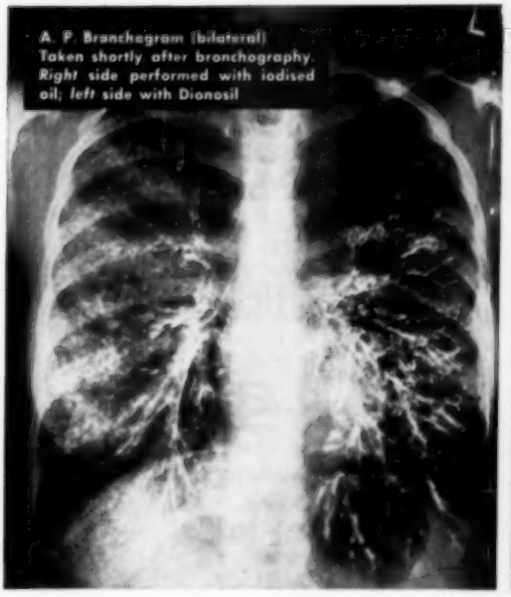
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THE EFFECT OF ISONIAZID ON LIPIDS OF THE TUBERCLE BACILLUS[1, 2]

HENRY P. RUSSE AND WILLIAM R. BARCLAY

(Received for publication May 24, 1955)

INTRODUCTION

Tubercle bacilli isolated from the animal host or grown on synthetic media exhibit an acid-fast staining character. It has been suggested that this quality is due in some way to the lipid composition of the organisms. In particular, mycolic acid has been implicated (1). Lamanna has proposed that acid-fastness is due to the relatively greater solubility of the dye in the lipids of the bacillus rather than in acid alcohol (2). Chouroum (3) has isolated a lipo-carbohydrate which is associated with both acid-fastness and the production of hypersensitivity. However, no complete explanation has been found to clarify the nature of acid-fastness which agrees with all the experimental data.

Recent investigations in this laboratory (4) have shown that the tubercle bacilli lose their acid-fastness when exposed to isoniazid. This loss of acid-fastness occurs only if the bacilli are actively metabolizing in the presence of isoniazid, and it does not occur if their metabolism is altered by other factors such as low temperature, deficient media, or previous exposure to other antituberculous drugs (8). Therefore, the loss of acid-fastness may reflect an altered metabolism which leads to a change in the normal lipid composition of the tubercle bacillus. To investigate this problem, the lipid composition of tubercle bacilli exposed to isoniazid was compared with the lipid composition of untreated organisms.

In an attempt to correlate acid-fastness with the production of hypersensitivity, the ability of isoniazid treated, nonacid-fast organisms to confer tuberculin hypersensitivity in guinea pigs was investigated and compared with the hypersensitizing ability of untreated acid-fast bacilli. Also tested in this respect were bacilli exposed to isoniazid at 6°C. and bacilli exposed to isoniazid after previous exposure to streptomycin.

[1] From the Department of Medicine, Division of Pulmonary Diseases, University of Chicago, Chicago, Illinois.

[2] Presented before the Medical Session, as part of *Section 3B*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 24, 1955.

MATERIALS AND METHODS

Avirulent tubercle bacilli, strain R1Rv, were grown on the surface of 500 ml. Proskauer and Beck liquid medium in extra wide bottom 2,500 ml. Erlenmeyer flasks. Eight flasks were inoculated with 0.1 mg. (wet weight) (5) of tubercle bacilli and incubated at 37°C. When the cultures were four to five weeks old, 10 γ of C^{14} labeled isoniazid (6) per ml. of medium were added to four of the flasks.[3] One week to ten days after the addition of the isoniazid, the bacilli were harvested and washed with distilled water containing 0.02 per cent Tween®, then lyophilized for twenty-four to thirty-six hours. Control organisms from the flasks to which no drug had been added were treated in the same manner. The lyophilized organisms were weighed, and the lipids were then extracted following a modification of the method of Macheboeuf, Dieryck, and Stoop (7) (figure 1).

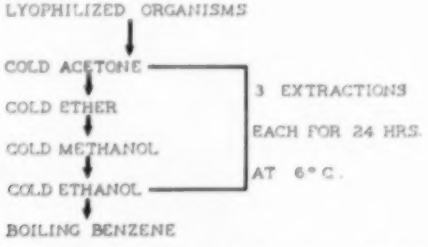

FIG. 1. Method for lipid extraction of tubercle bacilli (modified after Macheboeuf, Dieryck, and Stoop).

For the hypersensitivity experiments with guinea pigs, virulent tubercle bacilli, strain H37Rv, were grown in Dubos liquid medium. Erlenmeyer flasks (500 ml.) containing 100 ml. of medium were inoculated with 1.0 mg. (wet weight) of tubercle bacilli and incubated at 37°C. on a mechanical shaker to promote maximum aeration and rapid growth. The cultures of tubercle bacilli were divided into four groups and treated as follows:

Group I: Untreated culture

Group II: Twenty γ of isoniazid per ml. added after one-week incubation of the culture

Group III: After one week incubation, 20 γ of isoniazid per ml. added and the culture refrigerated at 6°C.

Group IV: Ten γ of streptomycin added per ml. of culture after one-week incubation, followed by the addition of 20 γ of isoniazid per ml. six days later.

Acid-fast stains were done on all groups of organisms ten days after the addition of the isoniazid. The organisms were pasteurized at 60°C. for thirty minutes and separated from the culture media by centrifugation and resuspended in sterile saline. A 0.2 ml. (containing approximately 4 mg. tubercle bacilli, wet weight) portion of the saline suspension was injected subcutaneously into the groin of guinea pigs which were not sensitive to Old Tuberculin (OT). Animals were challenged with 0.1 ml. of a 1:10 dilution of OT at two, three, four, and five weeks after the injections.

RESULTS

Effects of isoniazid on lipid composition of R1Rv: The lipid extracts were dried in a vacuum desiccator after separation from the bacilli by centrifugation. The extracts were weighed, and their per cent of the total dry weight of the organisms

[3] The distribution of C^{14} labeled isoniazid in the lipids will be reported in a subsequent paper.

TABLE 1

COMPARISON OF LIPID FRACTIONS OF R1Rv GROWN ON PROSKAUER AND BECK LIQUID MEDIUM WITH 10 MG. PER ML. OF ISONIAZID ADDED TO THE TREATED ORGANISMS

Experiment	Per Cent Dry Weight of Lipid Extract										Total Lipids Per Cent Dry Weight	
	Acetone		Ether		Methanol		Ethanol		Benzene			
	Control	Isoniazid-Treated	Control	Isoniazid Treated	Control	Isoniazid Treated	Control	Isoniazid Treated	Control	Isoniazid Treated	Control	Isoniazid Treated
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2.05	1.65	0.34	0.51	9.76	4.72	1.15	0.88	1.61	0.81	14.91	8.57
2	2.84	2.68	1.01	0.93	8.27	6.01	1.34	1.26	4.56	3.71	18.02	14.59
3	2.17	1.60	0.89	0.74	8.56	6.51	0.95	1.21	—	—	—	—
Average	2.35	1.97	0.75	0.73	8.86	5.74	1.14	1.11	3.08	2.26	16.47	11.59

TABLE 2

RESPONSE TO CHALLENGE WITH OLD TUBERCULIN (OT) AFTER INOCULATION WITH ACID-FAST PASTEURIZED H37Rv AND WITH NONACID-FAST PASTEURIZED ISONIAZID TREATED H37Rv

Guinea Pigs

Inoculated with	Challenged with OT 1:10 After		
	3 weeks	4 weeks	5 weeks
Untreated acid fast H37Rv	+ + +	+ + +	+ + +
	+ + +	+ + +	+ + +
	+ + +	+ + +	+ + +
	+ + + +	+ + +	+ + +
Isoniazid-treated nonacid fast H37Rv	0	0	0
	0	0	0
	0	0	0
	0	+	0

+ + + +—Necrosis. + + +—Severe reaction. + +—Moderate reaction. +—Minimal reaction. 0—No reaction.

was calculated. Although a higher per cent of lipids was extracted from the control group of organisms by each of the solvents, the most marked difference was seen in the methanol fraction. The values obtained are shown in table 1.

The effect of isoniazid on H37Rv in the production of hypersensitivity: When acid-fast stains were made ten days after the addition of isoniazid to the various cultures of tubercle bacilli, the control organisms (*Group I*), the organisms exposed to isoniazid at 6°C. (*Group III*), and the organisms treated with isoniazid after previous exposure to streptomycin (*Group IV*) were all found to be acid-fast. The tubercle bacilli treated with isoniazid alone (*Group II*) were more than 95 per cent nonacid-fast.

While the guinea pigs inoculated with the acid-fast control organisms (*Group I*) showed a marked hypersensitivity to tuberculin, the animals inoculated with

TABLE 3

RESPONSE TO CHALLENGE WITH OLD TUBERCULIN (OT) AFTER INOCULATION WITH ACID-FAST PASTEURIZED H37Rv TREATED WITH ISONIAZID AT 6°C. AND TREATED WITH ISONIAZID AFTER PREVIOUS EXPOSURE TO STREPTOMYCIN

Guinea Pigs

Inoculated with	Challenged with OT 1:10 After		
	2 Weeks	3 Weeks	4 Weeks
Treated with streptomycin and isoniazid acid fast H37Rv	+	++	+++
	+	++	+++
	++	+++	+++
Refrigerated, isoniazid-treated, acid-fast H37Rv	+	+++	++++
	+	+	++++
	+	+	++++
	+	+	+++
	0	±	++

++++—Necrosis. +++—Severe reaction. ++—Moderate reaction. +—Minimal reaction. 0—No reaction.

the nonacid-fast, isoniazid-treated organisms showed no response to challenge with OT (table 2).

Animals which had been inoculated with acid-fast organisms treated with both streptomycin and isoniazid (*Group IV*) showed a moderate reaction to challenging doses of OT. The guinea pigs inoculated with the acid-fast organisms, treated with isoniazid at 6°C. (*Group III*) responded minimally to challenging doses of OT at two and three weeks, but responded strongly to OT at four weeks after a delayed emergence of hypersensitivity (table 3).

DISCUSSION

It is concluded from these studies that one of the effects of isoniazid on the tubercle bacillus is the quantitative reduction of substances soluble in organic solvents, particularly methanol. The chemical nature of these lipid extracts is yet to be studied. They may possibly be similar in composition to those described by Macheboeuf and his co-workers. It is possible that the loss of acid-fastness is associated with this reduction of lipids in isoniazid-treated bacilli.

Exposure of growing virulent tubercle bacilli to high concentrations of isoniazid alters the ability of these organisms to confer hypersensitivity to tuberculin in guinea pigs. This possibly is a parallel phenomenon to the loss of acid-fastness.

The association of acid-fastness with the ability to confer hypersensitivity in susceptible animals has been suggested by Choucroun (3), who has isolated a lipo-carbohydrate from tubercle bacilli which is related to both of these qualities. She has cited the earlier work of Boissevain and Schaefer in support of her thesis. Boissevain and Schaefer were able to obtain nonacid-fast tubercle bacilli by a mild hydrolysis with hydrochloric acid. Subsequently, when such bacilli were injected into test animals, it was noted that they had lost the ability to sensitize the animals to tuberculin.

The present experimental results, in conjunction with the previously reported effects of isoniazid on the loss of acid-fastness of the tubercle bacillus, suggest that isoniazid forces growing tubercle bacilli into an altered metabolic pathway which is incompatible with the physiologic life of the organism. Possibly as a result of this altered metabolism, certain defects in lipid composition become apparent, as well as changes in staining character and alterations in the antigenicity of these organisms.

SUMMARY

Tubercle bacilli exposed to isoniazid *in vitro* show a decrease in the amount of lipids which may be extracted with organic solvents, particularly methanol, when compared with untreated tubercle bacilli.

Animal studies show that isoniazid-treated nonacid-fast tubercle bacilli lose the ability to confer hypersensitivity to tuberculin in guinea pigs.

SUMARIO

El Efecto de la Isoniacida sobre los Lípidos del Bacilo Tuberculoso

Al compararlos con bacilos tuberculosos no tratados, los bacilos tuberculosos expuestos *in vitro* a la isoniacida revelan una disminución en la cantidad de lípidos que puede extraerse con solventes orgánicos, y en particular metanol.

Los estudios en animales muestran que los bacilos tuberculosos anácidorresistentes tratados con isoniacida pierden la capacidad para crear hipersensibilidad a la tuberculina en los cobayos.

RESUME

L'action de l'isoniazide sur les lipides du bacille tuberculeux

Par comparaison avec les bacilles non traités, les bacilles tuberculeux exposés *in vitro* à l'isoniazide montrent une réduction de la quantité de lipides pouvant être extraits par les solvants organiques, en particulier par le méthanol.

Les études sur l'animal montrent que les bacilles tuberculeux non acido-résistants traités par l'isoniazide perdent leur capacité de conférer au cobaye l'allergie à la tuberculine.

REFERENCES

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COMPARISON OF THE EFFECT OF FOUR VARIABLES IN THE ANTIMICROBIAL THERAPY OF PULMONARY TUBERCULOSIS[1]

I. Report of the Cooperative Study of the Veterans Administration, Army, and Navy, April, 1949, to January, 1951

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INTRODUCTION

A cooperative program for the evaluation of variables in the antimicrobial therapy of tuberculosis was established in hospitals of the Veterans Administration, Army, and Navy in May, 1946. Seven hospitals participated originally; nearly fifty hospitals have participated in recent years.[4] This cooperative study has been one of three large cooperative studies concerned with the problems of the antimicrobial therapy of tuberculosis. In broad outline, these studies have been comparable with those conducted throughout the same years by the U. S. Public Health Service (19) and the British Medical Research Council (17, 18, 20, and 21).

Summaries of the earlier phases of the investigations by the Veterans Administration, Army, and Navy for the period 1946–1949 have been published (1–6). They have covered the period before para-aminosalicylic acid (PAS) was in general use. In brief, the findings in pulmonary tuberculosis were as follows (6):

> The administration of 2 gm. of streptomycin daily was associated with a high degree of toxicity which was avoided to a considerable extent when the dose was reduced to 1 gm. or 0.5 gm. daily, or to 1 gm. twice a week.
>
> The daily administration of 1 gm. of streptomycin was judged to be as effective therapeutically in pulmonary tuberculosis as 2 gm. of streptomycin daily. The 0.5 gm. daily dose was approximately as effective, but only slightly less toxic than the 1 gm. daily dose.
>
> The administration of 1 gm. of streptomycin daily in a single dose was as effective as 1 gm. in five divided doses (0.2 gm. every four hours from 8 a.m. to midnight), with no detectable difference in toxicity.
>
> The daily administration of streptomycin alone, whether at the 2, 1, or 0.5 gm. daily dosage, was associated with a high rate of emergence of tubercle bacilli resistant to streptomycin in concentrations of 10γ per ml. or higher *in vitro*.

[1] Portions of this report were presented to the Tenth Conference on the Chemotherapy of Tuberculosis (Streptomycin Conference), Atlanta, Georgia, January 25–28, 1951.

[2] Prepared by the writer at the request of the Executive Committee of the Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy.

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[4] The names of the hospitals which have participated in the cooperative study are listed under acknowledgments at the end of the article.

The administration of 1 gm. of streptomycin every third day or twice a week appreciably lowered the rate of emergence of streptomycin-resistant strains of tubercle bacilli.

Shortening the course of daily streptomycin therapy from four to two months or to forty-two days decreased both drug toxicity and the appearance of drug-resistant tubercle bacilli, but at a considerable cost in therapeutic efficacy. Relapse was more common than with regimens of four months' duration.

Dihydrostreptomycin, on the basis of preliminary reports, appeared to be as efficacious as streptomycin, with less toxicity for the vestibular nerve but more for the auditory nerve.

Preliminary reports, based on small-scale investigations, available in 1949, indicated that the multiple-drug regimen of streptomycin and para-aminosalicylic acid (PAS) offered much promise. This was apparent not only from the standpoint of the usual measures of therapeutic efficacy, such as roentgenographic improvement, reversal of infectiousness, and avoidance of relapse, but particularly from the standpoint of avoidance or delay in the rate of emergence of streptomycin-resistant strains of tubercle bacilli (7–9).

The present report will summarize briefly the findings of the cooperative study for the period 1949–1951, when streptomycin–PAS was being further tested, when the daily administration of streptomycin was being compared with so-called "intermittent" administration (every third day or twice a week), when a further comparison was being made of streptomycin and dihydrostreptomycin, and when regimens of three and four months' duration were being compared. Subsequent reports will deal with later periods of the cooperative study.

PLAN OF INVESTIGATION

Methods

Criteria for selection of patients: The following were requirements for admission of patients with pulmonary tuberculosis to the investigation (earlier requirements that there be a mandatory pretreatment observation period of sixty days before the start of antimicrobial therapy and that there be a life expectancy of at least one year were discontinued in 1948):

1. Demonstration of tubercle bacilli in sputum or gastric contents (all patients not meeting this requirement were excluded).
2. Progressive or stationary disease in a pretreatment observation period (the disease in the cases here reported proved to be progressive in 84 per cent, stationary in 16 per cent).
3. A "predominantly exudative component"[8] preferred (this proved to be present in 70 per cent in this series).

[8] Provisions of the protocol for selection of patients included the phrase "The X-ray will disclose some exudative component, the more the better." Physicians in reporting cases were asked to indicate whether in their opinion this exudative component constituted more or less than 50 per cent of the disease visible on the roentgenogram, if it constituted more than 50 per cent, it was designated as "predominantly exudative." It was recognized that estimation of a true exudative component by roentgenographic interpretation has its limitations. The provision with respect to the exudative component was discontinued in January, 1951, at the end of the period covered in this report.

4. Degree of bed rest in effect prior to antimicrobial therapy to remain unchanged during therapy and for four additional months.
5. Ipsilateral artificial pneumothorax not to be present at start of antimicrobial therapy; all forms of collapse therapy and other surgery permitted during and after antimicrobial therapy.[8]
6. Susceptibility of tubercle bacilli in 10 γ of streptomycin per ml. *in vitro* (patients with strains of tubercle bacilli which did not meet this criterion were excluded.)[7]

Method of assignment of regimen: After determination that a patient with pulmonary tuberculosis newly admitted to one of the participating hospitals met the criteria for inclusion in the study, determination as to which drug regimen would be employed was made at the hospital according to the last digit of the hospital register number.[8] Checks made of the impartiality of this method of random allocation have shown it to be unbiased (10).

Laboratory methods: Standardized laboratory methods for bacteriologic examinations, including drug-susceptibility determinations, are recommended to the participating hospitals by a Bacteriology Committee, assuring a considerable degree of uniformity of results among the participating hospitals (11).

Collection of data: Data on characteristics of the patient, his tuberculous disease, the details of his antimicrobial therapy, other therapy received, and observations on results are reported on standard individual case report forms (12) and are forwarded at intervals to the Reports and Statistics Service of the Veterans Administration (formerly the Medical Statistics Division), in Washington, D. C., for coding, compiling, and analysis. Resulting tables are published at conferences held at regular intervals (semiannually from 1946 to 1949; annually thereafter) (13). Observations are reported at the following intervals after the initiation of antimicrobial therapy: four, eight, twelve, eighteen, and twenty-four months. Roentgenographic change is interpreted by investigators at the individual participating hospitals, on a seven-point "scale," from "marked improvement" to "marked worsening" (6). A study of the objectivity of such roentgenographic interpretation, made by a panel of expert clinicians in April, 1948, revealed that there is essentially the same degree of agreement between hospital staff members and such experts as among the experts themselves when interpreting roentgenographic change on serial films on a completely objective basis (14).

[6] In this report results have not been analyzed of the role which the employment of collapse therapy or surgery may have played in the results observed. Data are available whether patients at each observation point had received (*a*) no collapse therapy or thoracic surgery, (*b*) pneumoperitoneum or phrenic nerve crush, or (*c*) "pneumothorax, thoracoplasty, resection, or other thoracic surgery." Of the 813 patients observed eight months after the start of therapy, for example, the percentages for (*a*), (*b*), and (*c*) were 25, 48, and 27, respectively. Of the 571 patients observed twelve months after the start of therapy, the percentages were 25, 45, and 30, respectively. At both observation points the percentages of roentgenographic improvement and of reversal of infectiousness were higher by approximately 15 points in groups (*b*) and (*c*) than in group (*a*). In no instance was there a significant difference, however, between regimens or groups of regimens as to the frequency or time of employment of collapse therapy or thoracic surgery.

[7] Of patients otherwise eligible for inclusion in this study, 14 per cent were excluded because of *in vitro* resistance to 10 γ of streptomycin (or dihydrostreptomycin) per ml.

[8] The provision has applied since the inception of the study that a seriously ill patient, even though meeting the criteria for selection of cases, may be withheld from the study. Furthermore, it has also been understood that a patient with a condition deteriorating under the treatment assigned by random allocation may at the physician's discretion be changed from that assigned to one of the physician's choice. The number of patients withheld or withdrawn from the study has been very small, less than 5 per cent of those eligible, and has always been uniformly distributed among patients assigned to or eligible for the different regimens.

Evaluation of results: At stated intervals after the initiation of antimicrobial therapy, information on the following observations was reported on the individual case report forms: change from start of therapy in (*a*) patient's general condition, (*b*) patient's temperature, (*c*) patient's weight, (*d*) over-all roentgenographic change (*see* above), (*e*) change in lung cavities (if present), (*f*) unfavorable roentgenographic change, (*g*) bacteriologic status, both by microscopy and by culture, of sputum and/or gastric aspirates, (*h*) *in vitro* susceptibility of tubercle bacilli cultured from sputum or gastric contents, (*i*) survival of the patient, and (*j*) toxicity to the drugs employed. In the earlier stages of the cooperative investigation considerable reliance had been placed on such clinical signs as general condition, change in temperature, and change in weight. As experience was accumulated, however, it was found that these observations were of limited prognostic and discriminatory value, and they are not employed in the present study (15). Primary reliance in this report is placed on roentgenographic improvement, of all degrees or of moderate and marked degree, and on reversal of infectiousness, i.e., "conversion" to negative of the sputum or gastric cultures for tubercle bacilli. Differences in terms of percentages are assumed to be statistically significant at the 0.02 probability level based on a 0.90 confidence coefficient.

Planning and coordination of study: At each successive conference results are reviewed and plans made either for continuation or modification of the investigation in the light of those results. These decisions are made on the recommendation of a Committee on Plans (formerly the Central Office Study Units Committee), representative of the participating hospitals, and of consultants closely in touch with the project, and then are adopted or modified by the entire conference. Between annual conferences an Executive Committee meets two or three times a year, and a permanent secretary in the Central Office of the Veterans Administration is responsible for the detailed administration of the program. Interim progress reports, containing informal narrative accounts of the progress of the investigations, are collected from and circulated to the participating institutions at three-month intervals between conferences.

Materials

In table 1 are outlined the dates after which patients with pulmonary tuberculosis were admitted to the cooperative investigation of the different drug-regimen studies since the inception of the program. Those in the first five columns (through October, 1948) have been reported previously (6). The present report covers those regimens listed in the sixth and seventh columns (for April and November, 1949).

At the Seventh Streptomycin Conference, in April, 1949, the following regimens were adopted for investigation (*see* table 1):

	Number of Patients
1. SM/3: 1 gm. of streptomycin daily for three months	137
2. DHSM/3: 1 gm. of dihydrostreptomycin daily for three months	48
3. SM PAS/3: 1 gm. of streptomycin daily plus PAS[9] for three months	201
4. DHSM PAS/3: 1 gm. of dihydrostreptomycin daily plus PAS for three months	88
5. SM PAS/4: 1 gm. of streptomycin daily plus PAS for four months	97
6. DHSM PAS/4: 1 gm. of dihydrostreptomycin daily plus PAS for four months	57
7. SM2x/wk/4[10]: 1 gm. of streptomycin twice a week for four months	103
8. SM2x/wk PAS/4: 1 gm. of streptomycin twice a week plus PAS for four months	82

[9] PAS dosage in all instances consisted of 12 gm. daily, i.e., 12 gm. of PAS equivalent in 15 gm. of sodium para-aminosalicylate (NaPAS), given 5 gm. three times daily.

[10] 2x/wk: twice a week.

TABLE 1

CALENDAR OF INTRODUCTION OF NEW DRUG REGIMENS IN COOPERATIVE STUDY OF PULMONARY TUBERCULOSIS

Drug Regimens										
Drug and Dosage	Duration	Dates of Study*								
		December 1946	May 1947	October 1947	April 1948	October 1948	April 1949	November 1949	April 1950	January 1951
	days									
2.0 gm. SM† per day	120	x								
2.0 gm. SM per day	60		x							
1.0 gm. SM per day	120		x	x						
0.5 gm. SM per day	120			x	x					
1.0 gm. SM per day	42				x	x				
0.5 gm. SM per day	42				x	x				
2.0 gm. DHSM per day	42					x				
1.0 gm. SM twice weekly	120					x	x			
1.0 gm. SM per day	90						x			
1.0 gm. DHSM per day	90						x			
1.0 gm. SM per day + PAS‡	90						x			
1.0 gm. DHSM per day + PAS	90						x			
1.0 gm. SM per day + PAS	120						x	x	x	
1.0 gm. DHSM per day + PAS	120						x	x		
1.0 gm. SM twice weekly + PAS	120						x	x	x	
1.0 gm. SM per day + PAS	prolonged§								x	x
1.0 gm. SM twice weekly + PAS	prolonged§								x	x
0.5 gm. SM per day + PAS	prolonged§									x

* Drug regimen studied from date indicated at head of column above "x" until date of next column to right under which "x" for that regimen no longer appears.

† SM = streptomycin; DHSM = dihydrostreptomycin.

‡ PAS = para-aminosalicylic acid (dose uniformly 12 gm. per day).

§ Duration of treatment with these three SM-PAS regimens: "4, 8, 12, 18 or more months, at the discretion of the investigator."

The total number of patients included in this study is 813. Different groups of hospitals treated patients on different combinations of regimens, generally employing at least four of the above eight regimens concurrently. The method of random allocation employed assigned three hospital register numbers each to two regimens, and two register numbers each to two other regimens. At the Eighth Streptomycin Conference, in November, 1949, on the basis of preliminary reports, investigation of five of the eight regimens listed was discontinued, and patients admitted after that date were treated by random allocation only on regimens 5, 6, and 8. Because of this change and the study of different combinations of regimens in different hospitals, the number of cases assigned to the eight regimens differed as indicated above, although random allocation was followed in all instances.

Examination of the eight regimens listed will reveal that by suitable pairings of regimens it is possible to investigate the effect of the following variables:

A. Daily vs. "intermittent" administration of streptomycin (with daily PAS, administered for four months); regimen 5 (SM-PAS/4) *vs.* regimen 8 (SM2x/wk-PAS/4)

B. Single drug therapy vs. combined therapy: regimens 1 (SM/3), 2 (DHSM/3), and 7 (SM2x/wk/4) *vs.* regimens 3 (SM-PAS/3), 4 (DHSM-PAS/3), and 8 (SM2x/wk-PAS/4)

C. Streptomycin vs. dihydrostreptomycin: regimens 1 (SM/3), 3 (SM-PAS/3), and 5 (SM-PAS/4) *vs.* regimens 2 (DHSM/3), 4 (DHSM-PAS/3), and 6 (DHSM-PAS/4)

D. Three-month vs. four-month duration of therapy: regimens 3 (SM-PAS/3) and 4 (DHSM-PAS/3) *vs.* regimens 5 (SM-PAS/4) and 6 (DHSM-PAS/4)

Such regimens may be compared and analyzed by pairs or by groups of pairs as long as individual paired analysis does not reveal statistically significant differences. In the presentation of results to follow, the latter procedure has been observed, it having been determined that no error is thereby introduced.

In table 2 may be seen characteristics of patients and their disease in the several grouped regimens. Not only is an indication given of the composition of the different treatment groups, but also measurement of their comparability is possible.

Of the entire group of 813 patients, 79 per cent had far advanced pulmonary tuberculosis; in 84 per cent the disease was progressive during the observation period immediately prior to antimicrobial therapy; the disease was bilateral in 79 per cent and cavernous in 78 per cent; 25 per cent were Negroes and 75 per cent were whites; 19 per cent of the patients had previously had antituberculous antimicrobial therapy, although the organisms of all were susceptible to 10 γ of streptomycin per ml. *in vitro*.

Supplementary collapse therapy: In addition, data not shown in table 2 are available to indicate that approximately 75 per cent of all the patients received some form of collapse therapy during antimicrobial therapy or within four months thereafter without significant difference in the different treatment groups.

Comparability of defining characteristics: As is indicated in table 2, there is good comparability of defining characteristics in the four paired comparison groups. Dagger marks (†) in the table indicate where differences are statistically significant. A previous study of the effect of variables listed as "defining characteristics" (16) has indicated the following:

Far advanced disease improves less than moderately advanced disease.

Progressive disease improves more than stationary disease.

Bilateral disease improves less than unilateral disease.

Patients with larger cavities improve less than those with smaller cavities or with no detectable cavities.

The disease responds approximately equally well in whites and in non-whites.

The disease in patients with prior antimicrobial therapy responds less well than in those without prior therapy, even though tubercle bacilli are susceptible to 10 γ of streptomycin per ml. *in vitro*.

TABLE 2

DEFINING CHARACTERISTICS AT START OF THERAPY OF PATIENTS WITH PULMONARY TUBERCULOSIS TREATED ON VARIOUS DRUG REGIMENS, 1949-1950

	Comparison A Daily vs. Intermittent SM* (with Daily PAS, Treated for Four Months)		*Comparison B* SM/DHSM without PAS vs. SM/DHSM with PAS		*Comparison C* SM vs. DHSM (with or without PAS)		*Comparison D* SM/DHSM + PAS of Three Months' vs. Four Months' Duration		All Patients in Study
	Daily SM + Daily PAS	2x/wk§ SM + Daily PAS	Without PAS	With PAS	SM	DHSM	Three-Months' Duration	Four-Months' Duration	
Number of Cases	97	82	288	371	435	191	289	154	813
	Per cent								
Characteristics									
Far advanced	86	82	76	76	74 †	83	75	83	79
Progressive (pre-therapy)	92 †	79	76	70	76	73	70 †	86	84
Bilateral	82	89	77	80	77	80	77	82	79
Cavernous	79	72	78 †	71	78	76	80	75	78
Race: Negro	13 †	40	26	24	22	25	21	17	25
Previous antimicrobial therapy	24	23	15 †	23	22	19	22	19	19

* SM = streptomycin; DHSM = dihydrostreptomycin; PAS = para-aminosalicylic acid.

† Difference in percentages in adjoining columns statistically significant.

§ 2x/wk = twice a week.

In one instance in table 2 (Comparison *B*) the advantage to the group treated without PAS, with respect to prior antimicrobial therapy, is to a degree at least offset by the advantage to the group treated with PAS with respect to the percentage with cavities. Slight differences of statistical significance do exist in the composition of the different treatment groups, but the degree of comparability is believed to be sufficiently satisfactory to warrant comparisons based on the assumption that the groups are indeed comparable.

RESULTS

The results of the studies are summarized in table 3, according to roentgenographic improvement and bacteriologic findings. These data were reported at the four-month observation point to the Ninth Streptomycin Conference (13*b*), and at the eight- and twelve-month observation points to the Tenth Streptomycin Conference (13*c*).

Comparison of daily and "intermittent" streptomycin: As may be seen from Comparison *A* in table 3, there were no significant differences between patients treated with daily or with twice-a-week streptomycin in addition to PAS for four months with respect to all degrees of roentgenographic improvement, improvement of both moderate and marked degree, and to reversal of infectiousness. In the group of patients treated with daily streptomycin and daily PAS, there were less favorable results at twelve months after the start of therapy (eight months after discontinuation of therapy) than at four or eight months; whereas, in the group treated with streptomycin twice a week and daily PAS, the

results achieved at eight months were generally maintained to the twelve-month point. The differences between the two groups at the twelve-month point, however, are not statistically significant.

Comparison of single-drug therapy with combined therapy: Comparison *B* in table 3 indicates that, with respect to roentgenographic improvement, either of all degrees or of both moderate and marked degree, there is no statistically significant difference between patients treated with either streptomycin or dihydrostreptomycin alone or with either of these drugs administered together with daily PAS. This is true for each pair of regimens included in the comparison and for all three together. There is, however, a statistically significant difference in the reversal of infectiousness ("conversion" rate) in favor of the groups treated with combined therapy at the four-month observation point; and, in addition, the differences at eight and twelve months approach but do not quite reach statis-

TABLE 3

RESULTS OF ANTIMICROBIAL THERAPY OF PULMONARY TUBERCULOSIS

Time of Observation After Start of Antimicrobial Therapy	*Comparison A* Daily vs. Intermittent SM* (with Daily PAS, Treated for Four Months)		*Comparison B* SM/DHSM without PAS vs. SM/DHSM with PAS		*Comparison C* SM vs. DHSM (with or without PAS)		*Comparison D* SM/DHSM + PAS of Three Months' vs. Four Months' Duration	
	Daily SM + Daily PAS	2x/wk§ SM + Daily PAS	Without PAS	With PAS	SM	DHSM	Three Months' Duration	Four Months' Duration
Number of Cases								
4 months	68	63	388	431	551	251	368	94
8 months	97	82	288	371	435	193	289	154
12 months	42	46	236	275	310	128	229	60
All Degrees of Roentgenographic Improvement (Per Cent)								
4 months	74	82	61	68	65	59	64 †	72
8 months	80	82	69	67	67	65	61 †	80
12 months	62	76	65	63	62	57	59	66
Moderate Plus Marked Roentgenographic Improvement (Per Cent)								
4 months	52	53	36	42	42	36	40 †	54
8 months	62	61	46	48	48	50	44 †	62
12 months	45	61	52	45	42	42	42	50
"Conversion" of Sputum or Gastric Aspirates by Culture (Per Cent)								
4 months	53	52	27 †	37	34	29	33 †	51
8 months	49	53	36	43	42	37	39 †	50
12 months	37	53	42	48	45	37	46	44

* SM = streptomycin; DHSM = dihydrostreptomycin; PAS = para-aminosalicylic acid.

† Difference in percentages in adjoining columns statistically significant.

§ 2x/wk = twice a week.

tical significance. Thus, aside from considerations of drug susceptibility and resistance, not referred to in this report, there is some evidence that streptomycin or dihydrostreptomycin administered with daily PAS for three or for four months is more effective than either agent administered without PAS for the same period.

Comparison of streptomycin and dihydrostreptomycin: Comparison *C* in table 3 indicates complete therapeutic equivalence of the two drugs, according to the criteria listed. No statistically significant difference is present, and this applies to each paired regimen comparison as well as to the totals.

Comparison of therapies of three and of four months' duration: Comparison *D*, in table 3 indicates that therapy of four months' duration is definitely superior to that of three months' duration. This is true for both paired comparisons and for the combined regimens. The differences in all of the three observations are statistically significant at four and eight months after the start of therapy.

DISCUSSION

Other studies (17–19) have conclusively established the fact that streptomycin therapy is beneficial in the therapy of pulmonary tuberculosis although, when therapy is of four months' duration, the difference between streptomycin therapy and untreated controls lessens with the passage of time (18). A major disadvantage to daily therapy with streptomycin alone is the high rate at which streptomycin-resistant tubercle bacilli emerge, in effect limiting the effects of such therapy in an appreciable number of patients. Various devices have been explored to avoid streptomycin resistance, such as administering streptomycin in alternate weeks or alternate months (20, 21); varying the daily dose of streptomycin from 2 gm. a day to 1 gm. a day to 0.5 gm. a day (6); shortening the duration of therapy from four months to three months to forty-two days; and spacing the administration of streptomycin to 1 gm. every third day (6). None of these was appreciably successful in avoiding the emergence of streptomycin resistance while maintaining therapeutic efficacy. It was not until PAS was administered along with streptomycin that much progress was made in delaying the rate of emergence of streptomycin-resistant tubercle bacilli.

The present report from the cooperative study program of the antimicrobial therapy of tuberculosis of the Veterans Administration, Army, and Navy covers the transitional period from single-drug therapy to "combined therapy" with either streptomycin or dihydrostreptomycin and PAS. The success of the streptomycin-PAS combination was eventually to permit prolongation of effective antimicrobial therapy. Findings in the present report concerned with "combined therapy" are consistent with those of others (9, 20–23).

Patients were admitted to the present study who had had prior antimicrobial therapy for tuberculosis, although those with streptomycin-resistant organisms were excluded. It has been shown that prior therapy affects the therapeutic response unfavorably (16), and, therefore, that the inclusion of patients with such prior therapy is less satisfactory than limiting an investigation to previously untreated patients. The percentage who received prior drug therapy in the different treatment groups in the present study was comparable, however, and therefore no bias was introduced by its inclusion.

The construction of the present study permitted, in addition to the comparison of single-drug therapy and "combined" therapy, a further comparison of streptomycin and dihydrostreptomycin. The data here reported demonstrate the therapeutic equivalence of these two forms of the drug which has subsequently been confirmed by others (24–26). The question of relative toxicity of the two related antibiotics for the eighth cranial nerve is pertinent to their comparison. In this study the incidence of toxicity for the vestibular nerve, as measured by diminished response to caloric stimulation, was 21 per cent for patients treated with streptomycin and 15 per cent for those treated with dihydrostreptomycin. The incidence of impaired hearing of all degrees, determined audiometrically, was 25 per cent for those treated with dihydrostreptomycin and 18 per cent for those treated with streptomycin; of impaired hearing of moderate or marked degree, it was 10 per cent for dihydrostreptomycin-treated patients and 5 per cent for streptomycin-treated patients (27). These differences are all statistically significant.

A beginning was made in this study of an investigation into the effect of the variable of the duration of antimicrobial therapy, employing the principle of random allocation, by contrasting the results of therapy of three and of four months' duration. Even in this narrow range of variation, the results were clear-cut in favor of the longer duration, at least for short-range follow-up observation.

Thus, the present study, of possibly more than historic interest, based on what in 1955 is regarded as relatively short-term therapy (three to four months), narrowed the range of variables for further investigation by the method of random allocation of patients to different regimens serving as concurrent controls for each other. Dihydrostreptomycin was found to have no particular advantage over streptomycin; streptomycin–PAS therapy was found to be more effective than therapy with streptomycin alone, especially with respect to the delay of emergence of streptomycin-resistant organisms; and treatment for four months was found to be more efficacious than for three. Some of the initial concern, that twice a week streptomycin therapy (with daily PAS) might be less effective than daily administration of both streptomycin and PAS, was allayed. The basis thus was laid for the next phase of the cooperative investigation, initiated in April, 1950, of prolonged therapy with streptomycin–PAS. This later phase of the investigation will be the subject of a subsequent report.

SUMMARY

A total of 813 patients with bacteriologically proved pulmonary tuberculosis, with tubercle bacilli susceptible *in vitro* to 10γ of streptomycin per ml., were admitted in 1949 and 1950 by random allocation to an investigation of the following comparisons:

A. Daily *vs.* "intermittent" streptomycin, with daily PAS, for four months

B. Single-drug therapy (streptomycin or dihydrostreptomycin) *vs.* "combined therapy" (either of the same drugs with daily PAS)

C. Streptomycin *vs.* dihydrostreptomycin

D. Three-month therapy *vs.* four-month therapy

Analysis of defining characteristics showed the comparison groups to be essentially comparable.

As judged by roentgenographic improvement, of all degrees or of both moderate and marked degree, and by reversal of infectiousness ("conversion" to negative by culture of sputum or gastric aspirates) with follow-up observations up to twelve months after the start of therapy, the following conclusions may be drawn:

1. "Intermittent" streptomycin–PAS therapy is as effective as daily streptomycin–PAS therapy.
2. "Combined therapy," consisting of either streptomycin or dihydrostreptomycin with PAS, is slightly superior in its results to either of the two streptomycins alone, statistically significantly so in some respects.
3. Streptomycin and dihydrostreptomycin are therapeutically equivalent, although they differ in the incidences of their respective toxicities for the two branches of the eighth cranial nerve.
4. Antimicrobial therapy with either streptomycin or dihydrostreptomycin and PAS, both given daily for four months, is superior to such "combined therapy" administered for three months.

These studies, a part of a cooperative investigation among nearly fifty hospitals in the Veterans Administration, Army, and Navy, led to the formulation of a study of prolonged streptomycin–PAS therapy which will be the subject of a subsequent report.

SUMARIO

Comparación del Efecto de Cuatro Variables en la Terapéutica Antimacrobiana de la Tuberculosis Pulmonar. I. Informe Relativo al Estudio Cooperativo Llevado a Cabo por la Administración de Veteranos, el Ejército y la Armada, abril, 1949, a enero, 1951

Un total de 813 enfermos que tenían tuberculosis pulmonar comprobada bacteriológicamente, con bacilos tuberculosos susceptibles *in vitro* a 10 γ de estreptomicina por ml., fué aceptado en 1949 y 1950, por asignación al azar, para una investigación que comprendía las siguientes comparaciones:

A. La estreptomicina diaria comparada con la "intermitente", con ácido para-aminosalicílico (PAS) diariamente durante cuatro meses.

B. Terapéutica con una sola droga (estreptomicina o dihidroestreptomicina) comparada con una "terapéutica combinada" (una u otra droga con PAS diariamente).

C. Estreptomicina comparada con dihidroestreptomicina.

D. Terapéutica de tres meses comparada con terapéutica de cuatro meses.

El análisis de las características de definición reveló que los grupos tomados eran esencialmente comparables.

Juzgando por la mejoría radiográfica de todo género o por la de orden moderado o notable, y por la inversión de la infecciosidad ("viraje" a negatividad de los cultivos del esputo o de los aspirados gástricos), cabe sacar las siguientes conclusiones.

1. La terapéutica "intermitente" con estreptomicina-PAS es tan eficaz como la diaria con estreptomicina-PAS.
2. La "terapéutica combinada", comprendiendo ya estreptomicina o dihidroestreptomicina con PAS, es ligeramente superior a una u otra estreptomicina por sí sola, alcanzando esto en algunos sentidos importancia estadística.

3. La estreptomicina y la dihidroestreptomicina son equivalentes terapéuticamente, aunque discrepan en la incidencia de su respectiva toxicidad para las dos ramas del octavo par craneal.
4. La terapéutica antimicrobiana con ya estreptomicina o dihidroestreptomicina y PAS, administrada en ambos casos diariamente durante cuatro meses, es superior a la misma "terapéutica combinada" administrada durante tres meses.

Estos estudios, parte de una investigación cooperativa entre casi cincuenta hospitales de la Administración de Veteranos, del Ejército y de la Armada, condujo a la formulación de un estudio de la terapéutica prolongada con estreptomicina PAS, que formará el tema de una comunicación subsiguiente.

RESUME

Comparaison de l'effet de quatre variables dans la thérapie antibactérienne de la tuberculose pulmonaire: I. Rapport sur l'étude collective effectuée par l'Administration des Vétérans, Armée et Marine entre Avril 1949 et Janvier 1951

Un total de 813 patients atteints de tuberculose pulmonaire bactériologiquement confirmée, avec bacilles tuberculeux sensibles à 10 γ de streptomycine par ml. *in vitro*, ont été répartis au hasard dans les séries comprises dans l'investigation portant sur les comparaisons suivantes:

A. Administration quotidienne comparée à l'administration "intermittente" de streptomycine associée au PAS chaque jour pendant quatre mois.
B. Traitement par médicament unique (streptomycine ou dihydrostreptomycine) comparée au "traitement combiné" (l'un ou l'autre de ces antibiotiques plus le PAS chaque jour).
C. Streptomycine comparée à la dihydrostreptomycine.
D. Cure de trois mois par rapport à la cure de quatre mois.

L'analyse de la détermination des caractéristiques montrait que les groupes de comparaison étaient essentiellement comparables.

En se fondant sur l'amélioration radiologique de tous degrés, ou de degré modéré ou marqué, et les résultats négatifs de la culture des crachats ou des spécimens obtenus par lavage gastrique, on peut titer les conclusions suivantes:

1. Le traitement "intermittent" streptomycine PAS est aussi efficace que la streptomycine-PAS thérapie quotidienne.
2. Le "traitement combiné" consistant en streptomycine, ou en dihydrostreptomycine, plus le PAS, donne des résultats légèrement supérieurs à ceux obtenus avec l'une ou l'autre de ces deux streptomycines, ceux-ci sont à certains égards significatifs du point de vue statistique.
3. La streptomycine et la dihydrostreptomycine sont thérapeutiquement équivalentes, bien qu'elles diffèrent quant à l'incidence de leur toxicité respective pour la huitième paire crânienne.
4. Le traitement antibactérien avec la streptomycine, ou la dihydrostreptomycine, plus le PAS, tous deux administrés quotidiennement pendant quatre mois, est supérieur à ce même "traitement combiné" administré pendant trois mois.

Ces études, qui représentent une partie de l'investigation collective effectuée parmi prés de cinquante hôpitaux de l'Administration des Vétérans, Armée et Marine, ont conduit à l'élaboration d'une étude sur le traitement streptomycine-PAS prolongé qui sera le sujet d'un rapport ultérieur.

Acknowledgments

Data for the cooperative studies reported have been contributed by the staffs of the following hospitals: Fitzsimons Army Hospital (formerly Fitzsimons General Hospital),

Denver, Colorado; U. S. Naval Hospitals at Sampson, N. Y.,[1] and San Diego, California (formerly located at Corona, California); and the following Veterans Administration Hospitals and Centers: Albany, N. Y.; Albuquerque, N. M.; Alexandria, La.; Aspinwall, Pa.[1]; Atlanta, Ga.; Augusta, Ga.; Baltimore, Md.; Batavia, N. Y.; Brockton, Mass.; Brecksville, Ohio; Bronx, N. Y.; Brooklyn, N. Y.; Butler, Pa.; Castle Point, N. Y.; Chamblee (Atlanta), Ga.[1]; Columbia, S. C.; Coral Gables, Fla.; Dayton, Ohio; Dearborn, Mich.; Downey, Ill.; East Orange, N. J.; Excelsior Springs, Mo.; Fort Benjamin Harrison (Indianapolis), Ind.[1]; Fort Logan (Denver), Colo.[1]; Framingham, Mass.[1]; Hines (Chicago), Ill.; Houston, Tex.; Indianapolis (Cold Spring Road), Ind.; Kerrville (formerly Legion), Tex.; Livermore, Calif.; Long Beach, Calif.; Louisville, Ky.; Madison, Wis.; Martinsburg, W. Va.; McKinney (Dallas), Tex.; Memphis (Kennedy Medical Teaching Group Hospital), Tenn.; Memphis (Lamar), Tenn.; Minneapolis, Minn.; Newington, Conn.; New Orleans, La.; Oakland, Calif.; Oteen (Asheville), N. C.; Outwood, Ky.; Portland, Ore.; Richmond, Va.; Rutland Heights, Mass.; San Fernando, Calif.; Springfield, Mo.[1]; Staten Island (Halloran), N. Y.[1]; Sunmount, N. Y.; Temple, Tex.; Tucson, Ariz.; Vancouver, Wash.; Van Nuys, Calif.[1]; Walla Walla, Wash.; Waukesha, Wis.; West Haven, Conn.; Whipple, Ariz.; and Wood (Milwaukee), Wis. It is regretted that it is impossible to list the names of the individual investigators in these hospitals who have made the studies possible.

Data compiled from forms submitted by participating hospitals have been compiled by the following statisticians in the Veterans Administration Central Office, Washington, D. C.: Morton Robins (1946–1947), Jack Beckerman (1946), Howard Dunn (1948–1949), Hyman J. Doben (1949–1951), Dorothy G. Livings (1949–1954), and John H. Williams, Jr. (1953–1954); supervision was supplied by Morton Robins, Chief, Biometrics Section (1949–1954) and Rachel M. Jenss, Assistant Chief, Medical Research Statistics Division (1949), under the following chiefs: Paul M. Densen (Chief, Medical Research Statistics Division, 1947–1949) and Daniel I. Rosen (Chief, Reports and Statistics Service, 1949–1954). Each has made a valuable contribution in the often difficult field of integration of statistical and clinical philosophies in addition to indispensable service in the handling of complex and voluminous clinical data.

From its inception the study has been guided by a perceptive "committee" in the Central Office of the Veterans Administration, at first called the Streptomycin Committee (in recent years the Committee on the Chemotherapy of Tuberculosis) under the general supervision of Dr. John B. Barnwell, Chief and Director of the Tuberculosis Service. The secretary of the committee from its beginning has been Dr. Arthur M. Walker, who has supplied constant guidance and stimulation to the project and to its individual participants.

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COMPARATIVE EFFICACY OF THREE STREPTOMYCIN AND PARA-AMINOSALICYLIC ACID REGIMENS OF PROLONGED DURATION IN PATIENTS WITH PREVIOUSLY UNTREATED PULMONARY TUBERCULOSIS[1]

II. An Account of the Cooperative Investigation of the Veterans Administration, Army, and Navy, February, 1951, to January, 1952

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INTRODUCTION

For the past few years somewhat parallel cooperative investigations of the effects of variables of the antimicrobial therapy of tuberculosis have been conducted by the Public Health Service in the United States, by the Medical Research Council of Great Britain, and by the United States Veterans Administration, Army, and Navy. A previous report (1) has recapitulated the progress of the cooperative investigations of the latter group in the antimicrobial therapy of pulmonary tuberculosis between 1949 and 1951. It was established in these investigations that the use of streptomycin and para-aminosalicylic acid (PAS) together was more effective than the use of streptomycin alone, particularly with respect to avoidance or delay of the emergence of streptomycin-resistant strains of tubercle bacilli. On the basis of the results of these studies, it was possible to undertake an investigation of the relative merits of three different regimens composed of streptomycin and PAS, administered for periods considerably more prolonged than in the previous studies. The findings of this second set of studies comprise the present report.

As indicated in the previous report ((1), table 1), after the Ninth Conference on the Chemotherapy of Tuberculosis (in April, 1950), an investigation was commenced which compared the effectiveness of streptomycin, 1 gm. daily plus 12 gm. of PAS daily,[4] and streptomycin, 1 gm. twice a week plus 12 gm. of PAS daily. At the Tenth Conference (in January, 1951), the study was extended by the inclusion of a third regimen, streptomycin, 0.5 gm. daily plus 12 gm. of PAS daily. Data in this report are from the period when all three regimens were stud-

[1] Presented in part at the Fourteenth Conference on the Chemotherapy of Tuberculosis, Atlanta, Georgia, February 7–10, 1955; and at the Medical Session, as part of *Section 1A*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 23, 1955.

[2] Prepared by the writer at the request of the Executive Committee of the Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy.

[3] Chief, Medical Service, Veterans Administration Hospital, and Professor of Medicine, Duke University School of Medicine, Durham, North Carolina; member of the Executive Committee and Chairman of Committee on Plans, Veterans Administration, Army, and Navy Cooperative Study.

[4] The PAS dosage throughout this report is 12 gm. a day, i.e., 12 gm. of PAS equivalent in 15 gm. of sodium para-aminosalicylate (NaPAS), administered 5 gm. three times daily.

ied concurrently. Preliminary reports of this investigation have been published (2–6).

The mechanisms of construction of the study permitted, in addition to the inter-regimen comparisons, an evaluation of the variable of duration of combined streptomycin–PAS therapy and of certain variables other than those directly involved in the antimicrobial therapy.

MATERIALS AND METHODS

The methods for constructing the study have been outlined in detail in the preceding report (1). Criteria for selection of cases have remained essentially unchanged. In the present investigation, the previously untreated patients have been separated from those who had received prior drug therapy even though the strains of tubercle bacilli isolated from the latter were susceptible *in vitro* to streptomycin concentrations of 10 γ per ml. Only the patients who had received no antituberculous chemotherapy before admission to the study are included in the present report.

Assignment of the three streptomycin-PAS regimens to the newly admitted previously untreated patients in the participating hospitals was strictly by random allocation by the method previously described (1). The following regimens were assigned by this method:

	Number of Patients
SM 1 PAS: streptomycin, 1 gm. daily, plus 12 gm. of PAS daily	444
SM ½ PAS: streptomycin, 0.5 gm. daily, plus 12 gm. of PAS daily	458
SM2x/wk PAS: streptomycin, 1 gm. twice weekly plus 12 gm. of PAS daily	589
Total	1,491

In all instances the duration of therapy was prescribed by protocol to be for four, eight, twelve, eighteen, or more months, at the discretion of the investigator. The method of random allocation (1) assigned 3 of 10 possible final digits of hospital register numbers to each of the first two regimens, and 4 digits to the third. Twelve months after therapy was initiated, the number of patients observed on the three regimens were, respectively, 263 (30.3 per cent) on regimen 1; 263 (30.3 per cent) on regimen 2; and 341 (39.4 per cent) on regimen 3, a total of 867 patients. These proportions afford a check on the thoroughness of the random allocation.

The data forming the basis for the present report were collected from the forty-eight participating hospitals[a] in the fall of 1953, approximately one year and a half after the last patient was admitted to the study (in January, 1952). This permitted longer follow-up observations of the patients who received prolonged therapy than had been possible in the preceding report. These data are published in the *Transactions of the Thirteenth Conference on the Chemotherapy of Tuberculosis* (7). As the result of the method of random allocation of regimens described above, the duration of treatment and of follow-up observations of patients in the three treatment groups was identical.

Exclusions and Withdrawals from Study

Data received from the participating hospitals on individual case report forms were screened by the Reports and Statistics Service of the Veterans Administration, to *exclude* the following:

1. Those patients who did not complete at least three and one-half months of treatment as prescribed by protocol

[a] Hospitals which participated in the study are listed under acknowledgments at the end of the article.

TABLE 1

STATUS OF STREPTOMYCIN-PAS THERAPY AT TIME OF REPORTING, BY REGIMEN

Status of Antimicrobial Therapy	Regimen 1: 1 gm. of SM* daily + 12 gm. of PAS* daily	Regimen 2: 0.5 gm. of SM + 12 gm. of PAS daily	Regimen 3: 1 gm. of SM 2x/wk + 12 gm. of PAS daily	Totals for Three Regimens
Number of patients	444	458	589	1491
	Per cent			
Continuing	14	14	19	16
Discontinued for following reasons:				
Planned course completed	49	50	43	47
Death	3	2	1	2
Drug toxicity	4	2	3	3
Drug resistance	2	1	1	1
Irregular discharge	16	20	19	18
Other reasons	12	11	14	13
Totals	100	100	100	100

* SM = streptomycin; PAS = para-aminosalicylic acid.
2x/wk = twice a week.

2. Those patients whose sputum or gastric aspirates did not contain tubercle bacilli at the start of therapy, either on microscopy or culture, or only by culture, with the results of microscopy unknown.
3. A very few patients, presumably previously untreated and whose organisms were found to be resistant *in vitro* to 10 γ of streptomycin per ml.

Proportional numbers of patients were excluded from each of the three regimens on this basis.

In addition to patients *excluded* for the reasons given above, a certain number were *withdrawn* from the study after meeting the criteria listed and being admitted. In table 1 may be seen the status at the time of the final reporting with respect to the antimicrobial therapy, the percentage of patients still on such therapy, and the percentage in whom therapy had been discontinued, with the reasons for such discontinuance. Except for patients still continuing original antimicrobial therapy at the time of reporting and those indicated as "planned course completed," the remainder may be regarded as premature "withdrawals" from the study for the reasons cited. These withdrawals are distinct from the exclusions indicated above.

It will be seen in table 1 that 16 per cent of all of the 1,491 patients were continuing on the original therapy at the time of the report, 47 per cent had completed the planned course of antimicrobial therapy, and 2 per cent had had their therapy discontinued because of death. In the remaining 35 per cent, therapy was discontinued prematurely for the following reasons: drug toxicity (3 per cent), drug resistance (1 per cent), irregular discharge (18 per cent); and for a variety of other reasons, such as refusal of drug, change in therapy for a period of surgery, or transfer to another hospital (13 per cent).[8] There

[8] The protocol governing the treatment of these patients provided that therapy assigned by random allocation might be terminated at the discretion of the investigator in event of drug toxicity, the emergence of significant drug resistance *in vitro*, or definite deterioration of the clinical condition of the patient; and withdrawals for these reasons are reflected in table 1. The criteria for such withdrawals were not rigidly defined but, as the previous article (1) has indicated, the percentage of patients withdrawn or withheld from the study for such reasons was low.

TABLE 2

COMPARABILITY OF PATIENTS WITH PULMONARY TUBERCULOSIS TREATED ON THREE STREPTOMYCIN-PAS REGIMENS BY RANDOM ALLOCATION

Defining Characteristics at Start of Therapy	Regimen 1	Regimen 2	Regimen 3	Totals for Three Regimens
	1 gm. of SM* daily + 12 gm. of PAS* daily	0.5 gm. of SM + 12 gm. of PAS daily	1 gm. of SM 2x/wk. + 12 gm. of PAS daily	
Number of patients	444	458	589	1491
	Per cent			
Race: Negro	20	24	19	21
Sex: male	99	98	99	99
Age: 40 years and older	46	41	44	44
Far advanced disease	59	62	60	60
Progressive pre-therapy	81	78	78	79
Bilateral disease	73	76	78	76
Cavities 4+ cm.	15	17	15	16
Prior collapse therapy or surgery	17	16	20	18
Other factors associated with outcome of therapy				
Collapse or surgery after start of therapy	45	47	47	46
Died from tuberculosis	4	3	2	3
Died, nontuberculous disease	1	1	1	1

* SM = streptomycin; PAS = para-aminosalicylic acid.

was no significant difference among the three regimens with respect to percentages of discontinuance of therapy for these several reasons.

Comparability of Patients Treated on the Three Regimens

In table 2 are the data for the 1,491 patients to whom the three streptomycin-PAS regimens were assigned with respect to percentage on each regimen who possessed certain defining characteristics at the start of therapy. With respect to such factors as race, sex, age, extent of tuberculous disease, trend prior to initiation of therapy, number of lungs involved, cavitary status, and prior collapse therapy or surgery, there was no statistically significant difference between any pair of regimens. The data in the table also indicate the frequency with which collapse therapy or surgery was employed after the start of antimicrobial therapy and the mortality from tuberculous or nontuberculous causes.[7] Again, there is no statistically significant difference among the three regimens.

Evaluation of Results

For the period of this study, data were no longer collected for such clinical signs as changes in patient's general condition, temperature, and weight, which had been employed as criteria in earlier phases of the cooperative investigative program (1). Data on streptomycin and PAS toxicity also are not presented, although they are on record.

For purposes of the present analysis, the criteria for the evaluation of the effectiveness of the three regimens have been restricted to the following four:

[7] As a footnote to the published tables (7) indicates, patients who did not complete at least three and one-half months of therapy have been excluded, including those who died during that period. Thus the deaths reported in table 2 do not represent the total number of deaths since the start of therapy.

1. "Significant" roentgenographic improvement, defined as moderate plus marked degrees of improvement, excluding slight improvement.
2. Reversal of infectiousness ("conversion by culture"), as determined bacteriologically by culture of sputum or gastric aspirates (includes patients with negative findings both on culture and on microscopy and those negative by culture, with results of examination of smear and sputum unknown. If the sputum specimens were negative for tubercle bacilli on culture and positive by microscopy, the patient remained in the "infectious" category).
3. Unfavorable roentgenographic change, including those patients whose serial roentgenograms showed unfavorable change in the tuberculous disease after prior improvement ("roentgenographic relapse") as well as those whose disease deteriorated roentgenographically without such prior improvement. These results are reported cumulatively by the life-table method (8).
4. Rate of cavity closure: disappearance from view of tuberculous cavities previously present, by all available roentgenographic techniques, including tomography where pertinent; such rates were calculated by the life table method (8).

As in the previous studies, observations were made, tabulated, and analyzed at four, eight, twelve, eighteen, and twenty-four months after the initiation of antimicrobial therapy. The same criterion for statistical significance was employed: a 0.02 probability level based on a 0.99 confidence coefficient (1).

RESULTS

The results of this study will be presented in three parts: a comparison of the three streptomycin–PAS regimens for all patients admitted to the investigation; a comparison of the three regimens according to various defining characteristics, as subgroups of the total; and a comparison of the results of the antimicrobial therapy of patients treated with streptomycin–PAS, with respect to the influence of variables other than those directly related to the antimicrobial therapy.

Comparison of Three Streptomycin–PAS Regimens

The results of treatment for each of the three streptomycin–PAS regimens are given in figures 1 to 4. The data are expressed in terms of percentages of total cases observed at each point in time on the abscissa for "significant" roentgenographic improvement; for unfavorable roentgenographic change; for reversal of infectiousness ("conversion by culture"); and for rates of cavity closure, respectively. In all four figures none of the differences among the regimens at any observation point is statistically significant.

Comparison of Three Regimens According to Certain Defining Characteristics

The method of collection, tabulation, and analysis of data permitted cross-tabulation of the results according to the following defining characteristics of the patients: age (less or more than forty years); extent of disease (moderately or far advanced; minimal cases too few (4 per cent) for separate analysis by regimen); cavitary component (diameter of largest cavity, single or multiple cavities); and duration of therapy.

In figure 5, it may be noted that, with respect to *significant* roentgenographic improvement, there was no statistically significant difference among the three regimens in either of the age groups, less or more than forty. Analysis according

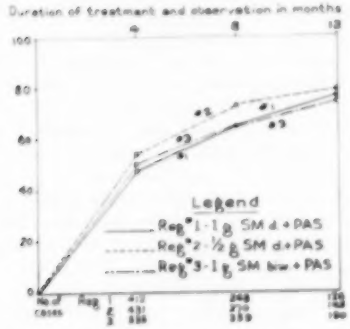

FIG. 1. Effect of regimen (three streptomycin-PAS regimens): I. On significant roentgenographic improvement. *(In this and the following figures (except figures 10, 11, and 12) the notation "Per Cent" was inadvertently omitted from the ordinate scale.)*

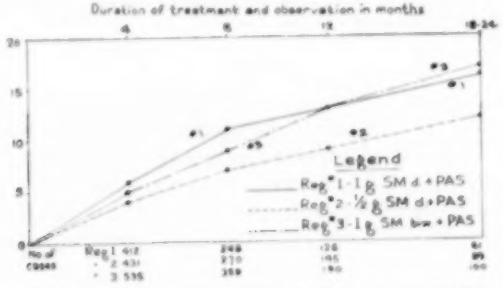

FIG. 2. Effect of regimen (three streptomycin-PAS regimens): II. On unfavorable roentgenographic change.

to the criteria of reversal of infectiousness ("conversion by culture") and unfavorable roentgenographic change showed a similar comparability of regimens in the two age groups.

In figure 6, it is demonstrated that for either moderately advanced or far advanced cases there was no statistically significant difference among the three regimens with respect to reversal of infectiousness ("conversion by culture"). The same comparability among regimens was present according to the criteria of *significant* roentgenographic improvement and unfavorable roentgenographic change.

It is demonstrated in figure 7 that for all single cavitary cases, regardless of size of cavity, there was no significant difference among the regimens with respect to unfavorable roentgenographic change. In figure 8 it may be noted that for single cavitary cases with the largest cavity of medium size (2.5 to 3.5 cm. in diameter) there was no significant difference according to reversal of infectiousness. In figure 9, for all cavitary cases (excluding those with no cavity demonstrable on roentgenographic observation), no significant difference is shown among the regimens for cavity closure rates.

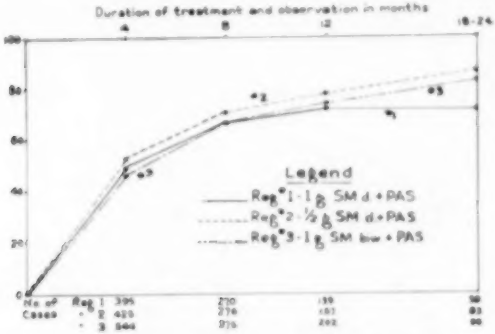

FIG. 3. Effect of regimen (three streptomycin-PAS regimens): III. On reversal of infectiousness (sputum or gastric aspirates) by culture.

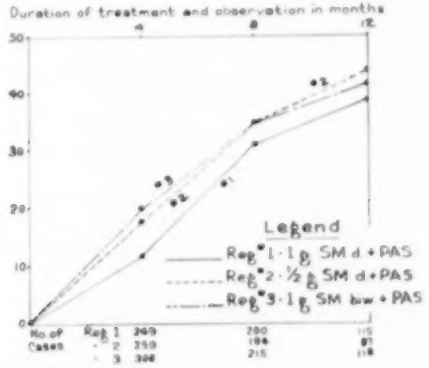

FIG. 4. Effect of regimen (three streptomycin-PAS regimens): IV. On rate of cavity closure in all cavitary cases (life-table method).

The comparisons among the three streptomycin-PAS regimens shown in the last five figures represent but a part of the total comparisons possible, according to various observations of results and defining characteristics. As is shown in table 3, comparisons have been made according to 48 combinations of observations and defining characteristics, at a total of 164 points in time. Thus, testing for inter-regimen differences of significance has been possible on 492 occasions, counting each inter-regimen comparison at each point in time for each criterion. Of these 492 comparisons, *only one* shows a statistically significant difference and that was of minor importance. For single cavity cases 2.5 to 3.5 cm. in diameter, those treated with 1 gm. of streptomycin plus 12 gm. of PAS daily had barely significantly more unfavorable roentgenographic change than those treated on the regimen of 0.5 gm. of streptomycin plus 12 gm. of PAS daily *at the four-month observation point only*, but not at other points in time. This detailed type of analysis permits the interpretation that the three streptomycin-PAS regimens

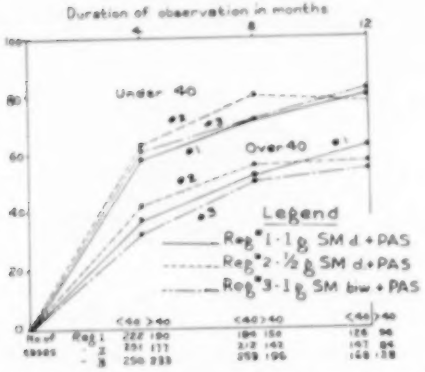

FIG. 5. Effect of regimen (three streptomycin-PAS regimens) on significant roentgenographic improvement: *A*, according to age.

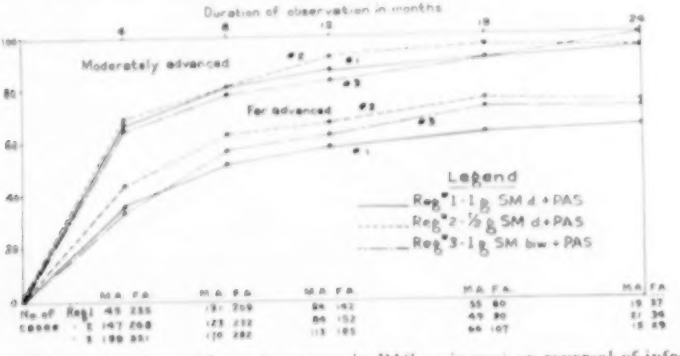
Duration of observation in months
Moderately advanced
Far advanced
Legend
Reg*1-1 g SM d + PAS
Reg*2-½ g SM d + PAS
Reg*3-1 g SM biw + PAS

FIG. 6. Effect of regimen (three streptomycin-PAS regimens) on reversal of infectiousness (sputum or gastric aspirates) by culture: *B*, according to extent of disease.

are therapeutically equivalent, according to the criteria employed, not only for all types of patients admitted to the study, but in all subgroups tested.

Comparisons of efficacy according to varying durations of antimicrobial therapy have been implied in the above observations. These have not been presented separately by regimen. As indicated in table 3, no statistically significant difference among the regimens could be detected for the treatment groups of different duration (four, eight, twelve, and eighteen months of therapy). Analysis of the effect of duration of antimicrobial therapy per se will be made below.

Effect of Variables Other than Those of Antimicrobial Therapy

The results of treatment with all three streptomycin-PAS regimens were added together and analyzed according to extent of disease, cavitary component, and duration of antimicrobial therapy. The procedure of combining the results for the three separate regimens has been justified by the detailed analysis al-

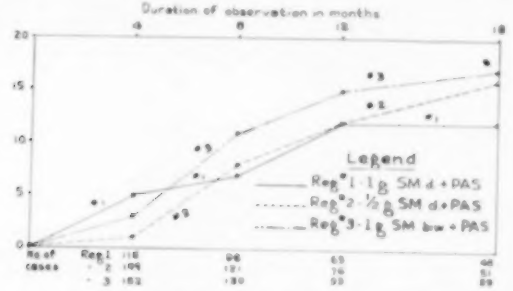

FIG. 7. Effect of regimen (three streptomycin-PAS regimens) on unfavorable roentgenographic change: *C*, according to cavitary component (in all single cavity cases).

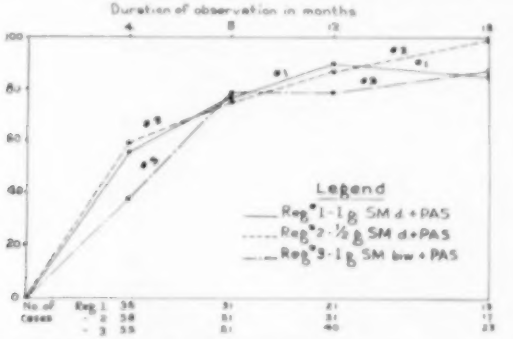

FIG. 8. Effect of regimen (three streptomycin-PAS regimens) on reversal of infectiousness (sputum or gastric aspirates) by culture: *C*, according to cavitary component (in all single cavity cases).

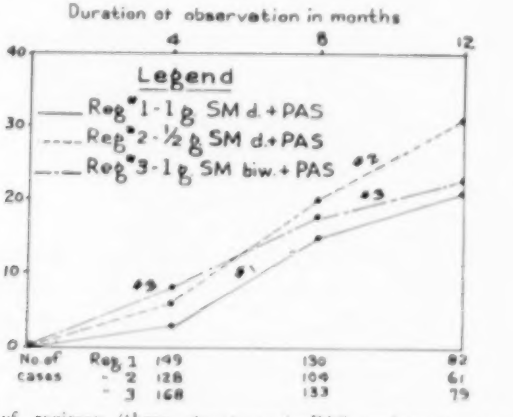

FIG. 9. Effect of regimen (three streptomycin-PAS regimens) on rate of cavity closure (life-table method): *C*, according to cavitary component in all cavitary cases.

TABLE 3

COMPARABILITY OF THREE STREPTOMYCIN-PAS REGIMENS (1951–1952 STUDY), ACCORDING TO VARIOUS FACTORS

Regimen 1—1 Gm. of Streptomycin per Day plus 12 Gm. of PAS per Day
Regimen 2—0.5 Gm. of Streptomycin per Day plus 12 Gm. of PAS per Day
Regimen 3—1 Gm. of Streptomycin Twice Weekly plus 12 Gm. of PAS per Day

Factors	Observation				
	Roentgenographic Improvement		Reversal of Infectiousness	Unfavorable Roentgenographic Change	Cavity Closure
	All Degrees	Moderate and Marked Degree			
I. Duration of Chemotherapy					
4 months of treatment	—	—	—	—	—
8 months of treatment	—	—	—	—	—
12 months of treatment	—	—	—	—	—
18 months of treatment			—	—	
II. Age					
Less than 40		—	—	—	
More than 40		—	—	—	
III. Extent of Disease					
Moderately advanced		—	—	—	
Far advanced		—	—	—	
IV. Cavitary Component					
No visible cavity		—	—	—	
Single cavity 0.5–2.0 cm.		—	—	—	—
Single cavity 2.5–3.5 cm.		—	—	+*	—
Single cavity 4.0 cm. and more		—	—	—	—
Multiple cavities		—	—	—	—

— No significant difference between regimens.
+ A significant difference between regimens.
* Regimen 1 exhibited barely significantly more unfavorable roentgenographic change than regimen 2 (not than regimen 3) at 4 months, but not at other points in time.

ready presented and, further, by testing each of these comparisons for each of the three streptomycin–PAS regimens separately. In no instance did the subanalysis by individual regimen differ in result from that for the three regimens combined.

Effect of extent of disease at start of therapy: In figures 10 to 12 the results are given for the three streptomycin–PAS regimens collectively with respect to *significant* roentgenographic improvement, unfavorable roentgenographic change, and reversal of infectiousness, respectively, for patients with minimal, moderately advanced, and far advanced disease at the start of antimicrobial therapy. In all three instances far advanced disease had the least favorable outcome; however, with respect to *significant* roentgenographic improvement the results were not significantly different in moderately advanced and in far advanced disease (figure 10). According to the other criteria (figures 11 and 12), however, unfavorable roentgenographic change and reversal of infectiousness,

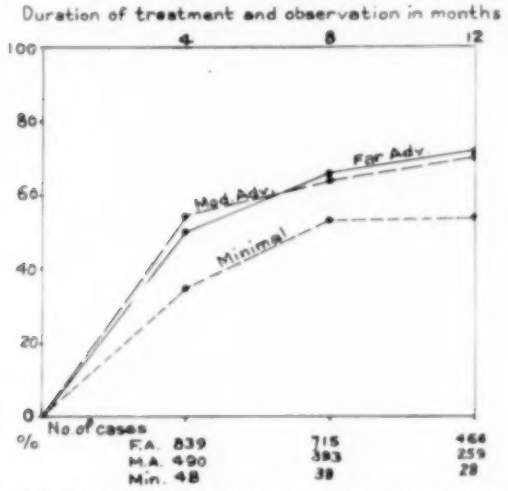

FIG. 10. Effect of extent of disease (three streptomycin-PAS regimens): I. On significant roentgenographic improvement.

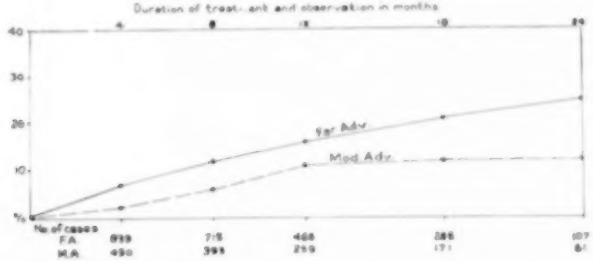

FIG. 11. Effect of extent of disease (three streptomycin-PAS regimens): II. On unfavorable roentgenographic change.

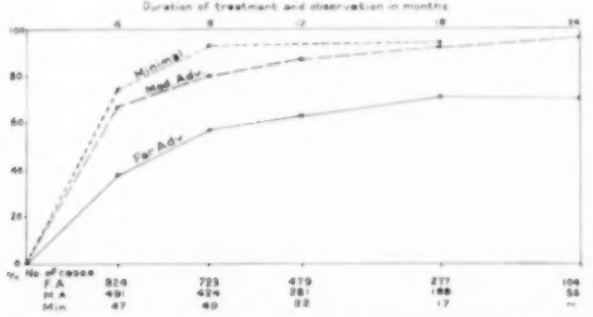

FIG. 12. Effect of extent of disease (three streptomycin-PAS regimens): III. On reversal of infectiousness (sputum or gastric aspirates) by culture.

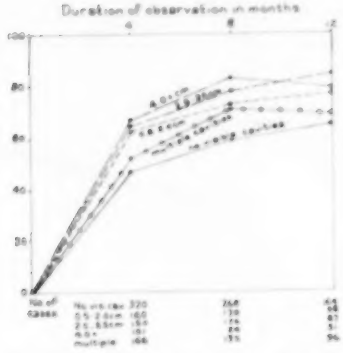

FIG. 13. Effect of cavitary component (three streptomycin-PAS regimens): I. On significant roentgenographic improvement.

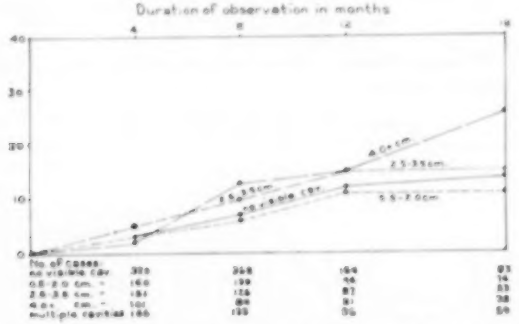

FIG. 14. Effect of cavitary component (three streptomycin-PAS regimens): II. On unfavorable roentgenographic change.

the results in far advanced disease were highly significantly less favorable than those for moderately advanced disease.

With respect to reversal of infectiousness (figure 12), the most objective of these criteria, it was noted that the minimal lesions improved most rapidly at first but that the rate of improvement of moderately advanced lesions approached that of minimal cases eighteen months after the start of therapy.

Effect of cavitary component: The results are presented in figures 13 to 16. In figure 13, for *significant* roentgenographic improvement, it will be noted that patients with no detectable cavities exhibited the lowest rate of improvement, those with multiple cavities the next lowest, and those with single cavities the most, the single cavitary cases with the largest diameter exhibiting the highest rates of improvement. The findings suggest that decrease in the size of cavities, particularly of the larger ones, is prominent in the interpretation of *significant* roentgenographic improvement.

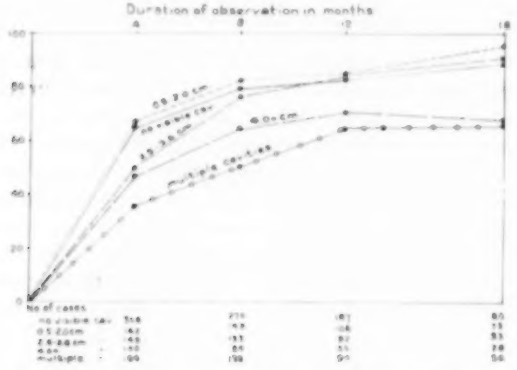

FIG. 15. Effect of cavitary component (three streptomycin-PAS regimens): III. On reversal of infectiousness (sputum or gastric aspirates) by culture.

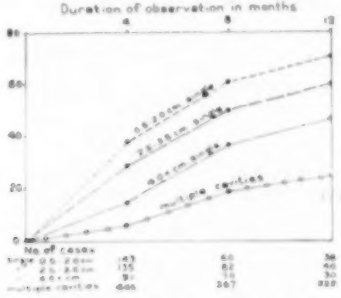

FIG. 16. Effect of cavitary component (three streptomycin-PAS regimens): IV. On rate of cavity closure (life-table method).

In figures 14 and 15 (unfavorable roentgenographic change and reversal of infectiousness, respectively), it is observed that patients with no detectable cavities and those with single cavities 0.5 to 2.0 cm. in diameter had approximately equal results at all observation points and better results than patients with more extensive cavitary disease. For reversal of infectiousness, patients with single cavities 2.5 to 3.5 cm. in diameter had rates approximating those with lesser cavitary components by eight months after the initiation of therapy, a finding which is consistent with that reported for patients with moderately advanced disease.

In figure 16 may be seen a clear-cut relationship between both size of cavitary component in single-cavity cases and multiplicity of cavities and rates of cavity closure by roentgenographic observation. The best results were observed in the smaller single cavitary cases. Thus, the majority of the evidence is to the effect that both the larger single cavity cases and cases of multiple cavities have less satisfactory results, as judged by the criteria employed.

Effect of Duration of Therapy

The effect of courses of streptomycin–PAS of varied duration was not investigated on the basis of allocation of regimens of different duration in a random fashion. In comparing the effects of treatment for varying lengths of time, therefore, it is necessary to establish the relative composition of the different duration of treatment groups with respect to as many defining characteristics as possible.

In table 4 the percentage distribution is given for seven characteristics of patients treated for periods from four to twenty-four months, varying in number from 501 to 44. The table is divided into characteristics which have previously been established (9) as having no clear association with the course of therapy (such as race and age), those having a favorable effect on the course of therapy (such as disease progressive in the pretreatment period), and those associated with relatively unfavorable progression of the disease (in general the variables of more advanced disease).

In general, it may be seen in table 4 that the distribution of such defining characteristics is not significantly different for the different therapy-duration groups with respect to those characteristics which have little association with outcome (race, age, and pretreatment collapse therapy or surgery). The composition of the therapy-duration groups with respect to the characteristic of pretreatment

TABLE 4

COMPARISON OF DEFINING CHARACTERISTICS OF PATIENTS TREATED WITH STREPTOMYCIN-PAS FOR DIFFERENT DURATIONS OF TIME

Defining Characteristics	Duration of Chemotherapy in Months					Differences Between Treatment Groups Significant
	4	8	12	18	24	
Number of patients	501	456	328	147	44	
	Per cent					
Characteristics not clearly associated with outcome of therapy						
Race: white	78	78	79	72	77	No
Age: more than 40 years	45	46	41	39	45	No
Collapse therapy or surgery before start of chemotherapy	20	17	15	14	21	No
Characteristics associated with favorable course						
Pretreatment trend: progressive disease	80	79	79	79	69	No
stationary disease	18	18	18	18	26	No
Characteristics associated with unfavorable course						
Stage: far advanced	56	58	67*	68*	75*	Yes
Bilateral disease	76	73	77	83*	86	Yes
Multiple cavities	38	34	40	56*	50	Yes

* Difference in per cent from four-month treatment group statistically significant.

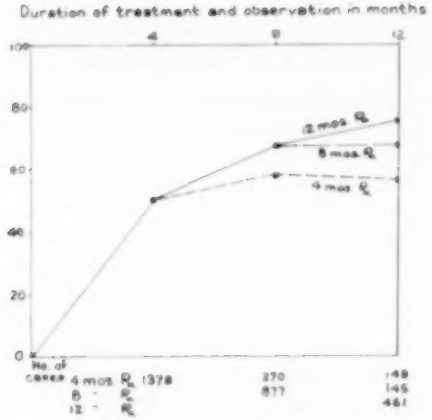

FIG. 17. Effect of duration of antimicrobial therapy (three streptomycin-PAS regimens): I. On significant roentgenographic improvement.

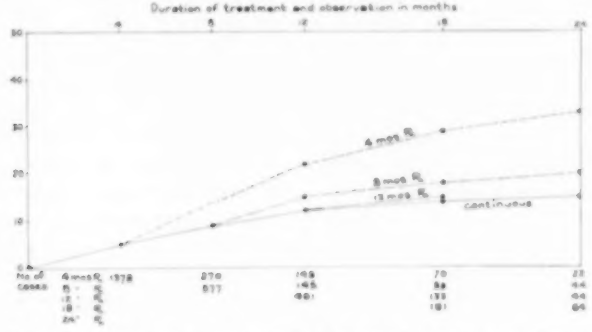

FIG. 18. Effect of duration of antimicrobial therapy (three streptomycin-PAS regimens): II. On unfavorable roentgenographic change.

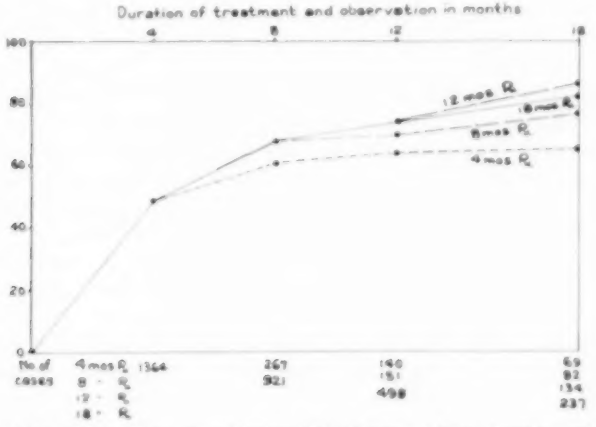

FIG. 19. Effect of duration of antimicrobial therapy (three streptomycin-PAS regimens): III. On reversal of infectiousness (sputum or gastric aspirates) by culture.

trend of disease (progressive or stationary) is remarkably constant (except for the small twenty-four-month-treatment group). Only with respect to those characteristics clearly associated with a less favorable outcome (extent of disease, bilaterality, and multiplicity of cavities), are there statistically significant differences. The trend in all three instances is clearly in the direction of longer duration of therapy for patients having more unfavorable characteristics. There appears to be good evidence, therefore, that those patients treated by the choice of the investigator for the longer periods had more unfavorable disease and poorer prognosis at the outset than those treated by choice for the shorter periods.

In figures 17 to 19 may be noted the results of streptomycin–PAS therapy of different durations according to the three criteria, *significant* roentgenographic improvement, unfavorable roentgenographic change, and reversal of infectiousness, respectively. All three figures are similarly constructed, with solid lines representing the longest durations of continuous original therapy and broken lines branching off after discontinuation at various intervals but with observation of results maintained after such discontinuance of therapy.

The differences observed between those patients who received only four months of therapy and those who received eight months of therapy, and between those who received eight and twelve months of therapy are in all instances statistically significant. For unfavorable roentgenographic change and for reversal of infectiousness (figures 18, 19), the differences between groups treated for twelve months and for longer periods are not statistically significant. There is evidence, therefore, that patients treated for longer duration, at least for twelve months (and possibly for eighteen or twenty-four months), in spite of having characteristics associated with more unfavorable prognosis, have results which are superior to those with more favorable defining characteristics but who received treatment for only four to eight months.

DISCUSSION

In the present study, in which the relative effectiveness of three streptomycin–PAS regimens is compared, the techniques of the cooperative investigation have been refined as compared with those of earlier periods. Data have been collected from participating hospitals for a uniform period when there was random allocation of patients to regimens for all hospitals for the entire period, i.e., treatment initiated between February, 1951, and January, 1952. Withdrawals have been uniformly distributed among the three regimens, and duration of observation of the three groups has been similarly uniform. These refinements in such cooperative investigations have led to a high degree of comparability of the treatment groups, as reflected by the data presented in table 2 and by the percentages of patients to whom the regimens have been assigned. The precision of the evaluation has been further increased by the exclusion of patients who received any prior antimicrobial therapy, even though *in vitro* tests indicated that their tubercle bacilli continued to be drug-susceptible. Conditions for a rigorous test of the regimens themselves, *as the sole variable*, thus appear to have been met.

Against this background of comparability of the three treatment groups, the

comparisons of results for the patients treated on the three streptomycin–PAS regimens may be accepted with considerable confidence. The interpretation can be made that, judged by the criteria employed—*significant* roentgenographic improvement, unfavorable roentgenographic change, reversal of infectiousness ("conversion by culture"), and cavity closure—the three regimens (1 gm. a day of streptomycin, 0.5 gm. a day, or 1 gm. twice a week, with 12 gm. of PAS a day in each instance) are all equally efficacious. Moreover, follow-up observations of these patients in an appreciable percentage for two years indicate equivalent mortality experience. It is possible, however, that employment of other criteria could modify these interpretations. For example, it has been suggested that, with respect to the culturability of tubercle bacilli from resected lesions, the organisms are less readily cultured when streptomycin has been administered to the patient daily rather than twice a week, with daily PAS in each instance (10). In the final analysis, a long-term follow-up of these patients probably will provide the best answer. Nevertheless, with follow-up observations for a two-year period in an appreciable number, there is no indication that the "intermittent" streptomycin-PAS regimen leads to results inferior to those obtained when the streptomycin is administered daily.

The detailed analysis comparing the three regimens by subgroups represents an attempt to ascertain whether the inclusion of certain more favorable cases of pulmonary tuberculosis (those less advanced, those with smaller cavitary components, et cetera) have concealed actual differences between regimens which might be apparent in less favorable cases. In effect, the analysis was designed to answer such a question as: "Granting that the three regimens have about the same effectiveness for all types of pulmonary tuberculosis, might it not be that for some kinds, perhaps the most advanced or the most cavernous, one of the regimens—for example, that combining 1 gm. of streptomycin and 12 gm. of PAS daily, for twelve or more months—is the best?" The analysis has shown, however, that, within the limits of the variables investigated, it cannot be stated that any one of the three regimens is therapeutically superior to either of the others, even for the most extensive disease. It is striking that, of the 492 comparisons listed in table 3, only one (0.2 per cent) is statistically significant, and this of itself could occur by chance.

That portion of this report concerned with variables other than those of antimicrobial therapy obviously compares noncomparable groups of patients, for random allocation was not involved as it was in the other two parts of the study. The results concerning the effect of the extent of disease and its cavitary component may be regarded, therefore, as reporting more or less "standard results" of therapy in patients who received their first course of antimicrobial therapy; and such results have a certain usefulness in the evaluation of certain treatment groups.

There is an interesting inconsistency between the results according to the criterion "significant" roentgenographic improvement, with respect either to stage of disease (figure 10) or cavitary component (figure 13) and the results according to the criteria reversal of infectiousness (figures 12 and 15) and cavity

closure rate (figure 16). According to the latter criteria, which are more objective than interpretation of roentgenographic change, the results are consistent with common clinical experience in that the more extensive the type of disease, either according to the National Tuberculosis Association classification of extent or to size of cavitary component, the poorer the result. With respect to the criterion *significant* roentgenographic improvement, however, there appears to be a reverse relationship: minimal disease improves less than moderately or far advanced disease (figure 10) and smaller cavitary cases improve less than larger cavitary cases (figure 13).

These observations suggest, not only that decrease in diameter of the larger cavities during effective antimicrobial therapy plays an important role in the interpretation of *significant*, i.e. moderate plus marked, roentgenographic improvement, but also that this criterion is less discriminatory in the evaluation of the effectiveness of drug regimens employed for long periods in the treatment of pulmonary tuberculosis. Thus, just as such clinical signs as temperature change, weight change, and change in general condition have been found to be of relatively little prognostic significance with effective prolonged antimicrobial therapy, so it may be that measures of improvement involving roentgenographic change may be of less significance after the first few weeks or months than such other measures as reversal of infectiousness, closure of cavities, relapse, and, ultimately, survival.

The portion of the present study concerned with the effect of duration of therapy upon outcome constitutes an attempt to compare treatment groups with differing durations of therapy when that duration has not been assigned by random allocation. Results have not yet been reported for any study of the duration of antimicrobial therapy employing random allocation of duration, except for that between durations of three and four months (1). A limited exploration of this question has been outlined by the Veterans Administration, Army, and Navy on a pilot-study basis (11), but the results are not yet available.

In the absence of random allocation, it is altogether likely that the different duration groups will differ in their constitution, and evidence to this effect has been presented. Somewhat fortuitously, inasmuch as duration of therapy was left to the determination of the individual investigator, evidence exists that those patients treated for the longer durations possessed characteristics more unfavorable to the outcome of their therapy than did those treated for shorter durations. Thus, if those patients treated for longer periods, in spite of more unfavorable characteristics, have results superior to those treated for shorter periods with more favorable characteristics, the interpretation may be made that antimicrobial therapy of such longer duration is in effect superior to that of shorter duration. This interpretation appears to be justified from the data herein presented.

The number of patients treated for more than twelve months is not sufficiently large to ensure the reliability of the difference in effectiveness between twelve, eighteen, and twenty-four months of therapy, but there is a suggestion that eighteen months of therapy approximate the optimal period. It may be further

noted that even this type of comparison of efficacy of differing durations of therapy is possible only when there are sufficiently large groups for each duration period. It is probable that, with the generally increasing duration of therapy for all patients with active pulmonary tuberculosis, the opportunity present in this 1951–1952 study, which represents a transition between the practices of relatively short and relatively long periods of therapy, will not again be available.

Comparison of the results of these investigations with those of other cooperative groups is not possible, as neither the Public Health Service in the United States nor the Medical Research Council trials in Great Britain has extensively investigated the comparative efficacy of daily streptomycin and daily PAS and twice-a-week streptomycin and daily PAS. The Public Health Service has employed the latter regimen in comparison with other regimens, especially since the introduction of isoniazid. In the Medical Research Council trials in Great Britain this regimen has been studied only as a continuation regimen, when clinicians have elected to reduce the dosage of streptomycin after an initial period of therapy with daily streptomycin and daily PAS. A recent report of the Medical Research Council trials has compared twice weekly streptomycin and daily isoniazid with daily streptomycin and daily isoniazid, and has indicated that the "intermittent" streptomycin dosage is less effective than the daily streptomycin regimen in preventing the emergence of drug-resistant strains of tubercle bacilli (12). This relationship between "intermittent" and daily streptomycin when administered with isoniazid, however, need not necessarily apply when streptomycin is administered with PAS.

Data have not been presented in the present report on drug resistance, but preliminary analysis of these data (13) does not indicate that the rates of emergence of streptomycin resistance differ significantly among the three streptomycin–PAS regimens. Data on drug resistance will be reported more fully at a later date.

SUMMARY

A controlled investigation has been conducted by cooperating hospitals in the Veterans Administration, Army, and Navy, of the relative efficacy of three streptomycin–PAS regimens, employing, respectively, 1 gm. of streptomycin daily, 0.5 gm. daily, and 1 gm. twice a week, with 12 gm. of PAS daily in each instance. The regimens were assigned by random allocation to 1,491 patients with bacteriologically proved pulmonary tuberculosis who had not received prior antituberculous chemotherapy, and the three treatment groups were found to be entirely comparable.

No significant differences in results of treatment on the three regimens were found with respect to roentgenographic improvement of moderate and marked degree; to unfavorable roentgenographic change; to reversal of infectiousness ("conversion" to negative by culture of sputum or gastric aspirates); or to cavity closure rates. This was true, not only for the entire group of 1,491 patients, but also for all subgroups analyzed according to such characteristics as duration of therapy, extent of disease, and size and number of tuberculous cavities.

Combining the results for the three separate streptomycin–PAS regimens, in view of the comparability of their results, it was found that there was a definite relationship between extent of the pulmonary tuberculous disease, as measured either by the National Tuberculosis Association classification, or by number and size of tuberculous cavities, and the outcome. As judged by reversal of infectiousness, unfavorable roentgenographic change, and cavity closure rates, the more extensive the disease the less favorable was the outcome. Judged by the criterion of both moderate and marked roentgenographic improvement, however, a reverse relationship was noted, with more improvement among those patients with more advanced disease. It has been suggested that this is related to the fact that rapid closing of the larger cavities has an unduly high influence in the interpretation of such *significant* roentgenographic improvement, and, therefore, this criterion is of less prognostic and discriminatory significance.

It has proved to be possible by suitable statistical techniques to compare the efficacy of periods of streptomycin–PAS therapy of varied durations even though this variable was not assigned to patients by random allocation. Evidence is presented that streptomycin–PAS therapy for a duration of at least twelve months is clearly superior to therapy of eight or four months' duration and that therapy of eighteen to twenty-four months is slightly, if not significantly, superior to that of twelve months' duration.

SUMARIO

Comparativa Eficacia de Tres Regímenes Prolongados de Estreptomicina y Acido Para-Aminosalicílico en el Tratamiento de la Tuberculosis Pulmonar en Enfermos que Recibieron Entonces su Primera Serie de Farmacoterapia: II. Reseña de la Investigación Cooperativa Llevada a Cabo por la Administración de Veteranos, el Ejército y la Armada, de febrero, 1951, a enero, 1952

Una investigación fiscalizada ha sido llevada a cabo en cooperación por hospitales de la Administración de Veteranos, del Ejército y de la Armada con respecto a la relativa eficacia de tres regímenes de estreptomicina y ácido para-aminosalicílico (PAS), en que se emplearon, respectivamente, 1 gm. diario de estreptomicina, 0.5 gm. diarios y 1 gm. dos veces semanales, con 12 gm. diarios de PAS en cada caso. Los regímenes se asignaron al azar entre 1,491 enfermos que padecían de tuberculosis pulmonar comprobada bacteriológicamente y que no habían recibido antes terapéutica antimicrobiana antituberculosa, y los tres grupos de terapéutica resultaron ser absolutamente comparables.

No se descubrieron mayores diferencias en los resultados del tratamiento con los tres regímenes en cuanto a: mejoría radiográfica de orden moderado o notable; alteraciones radiográficas desfavorables; inversión de la infecciosidad ("viraje" a negatividad en los cultivos del esputo o de los aspirados gástricos); o índices de clausura de cavernas. Esto fué un hecho, no sólo para todo el grupo de 1,491 enfermos, sino también para todos los subgrupos analizados con respecto a características tales como duración de la terapéutica, extensión de la enfermedad y tamaño y número de cavernas tuberculosas.

Combinando los resultados para los tres distintos regímenes de estreptomicina-PAS, vista la comparabilidad de aquéllos, observóse que existía una relación bien definida entre la extensión de la afección tuberculosa del pulmón, apreciada ya de acuerdo con la clasificación de la Asociación Nacional contra la Tuberculosis de los E. U. A. o por el número y el tamaño de las cavernas tuberculosas, y por el resultado. Juzgando por el "viraje de los cultivos", la alteración radiográfica desfavorable y los índices de cierre de cavernas, mientras más extensa era la dolencia, menos favorable era el resultado. Sin embargo, juzgando

por la pauta de mejoría radiográfica moderada y notable, se notó une relación inversa, con mayor mejoría entre los enfermos que padecían de afección más avanzada. Se ha sugerido que esto se enlaza con el hecho de que el rápido cierre de las cavernas más grandes ejerce un efecto exagerado en la interpretación de una mejoría radiográfica tan "importante" y, por lo tanto, esa pauta posee menos importancia diagnóstica y diferenciadora.

Ha resultado posible comparar, con una técnica estadística adecuada, la eficacia de períodos de duración variable de terapéutica antimicrobiana combinada con estreptomicina y PAS, a pesar de no haberse asignado esa variable a los enfermos por distribución al azar. Preséntanse pruebas de que esa terapéutica combinada aplicada a lo menos doce meses es netamente superior a la terapéutica de ocho o de cuatro meses de duración, y de que la terapéutica de dieciocho a veinticuatro meses es ligera, sino significativamente, superior a la de doce meses de duración.

RESUME

Efficacité comparée de trois cures de durée prolongée avec la streptomycine et l'acide para-aminosalicylique dans le traitement de la tuberculose pulmonaire chez des patients soumis pour la première fois à ces agents thérapeutiques. II. Un compte-rendu de l'investigation coopérative de l'Administration des Vétérans, Armée et Marine, de Février 1951 à Janvier 1952

Une investigation contrôlée a été effectuée avec le concours de divers hôpitaux de l'Administration des Vétérans, Armée et Marine, sur l'efficacité relative de trois cures par la streptomycine associée à l'acide para-aminosalicylique (PAS) en utilisant, respectivement, 1 g de streptomycine par jour, 0,5 g par jour et 1 g deux fois par jour plus 12 g de PAS par jour dans chaque cas. Les cures ont été assignées au hasard parmi 1.491 patients présentant une tuberculose pulmonaire confirmée bactériologiquement et n'ayant pas encore été traités par les antibactériens antituberculeux; les trois groupes ont été trouvés absolument comparables.

Il n'a été observé aucune différence significative entre les trois traitements en ce qui concerne l'amélioration de degré moyen ou marqué; les modifications défavorables du radiogramme; le renversement de l'infectiosité (la "conversion" des cultures de crachats et de tubage gastrique qui deviennent négatives); ou les taux d'oblitération des cavernes. Ceci était vrai, non seulement pour la totalité des 1.491 malades, mais aussi pour tous les sous-groupes analysés d'après certaines caractéristiques, telles que la durée de la cure, l'étendue de l'affection et les dimensions et le nombre des excavations tuberculeuses.

En combinant les résultats des trois cures streptomycine-PAS séparées, en vue de la comparabilité de leurs résultats, il est apparu qu'il existait une relation définie entre l'étendue des lésions tuberculeuses du poumon, évaluée par la classification de National Tuberculosis association ou par les dimensions et le nombre des excavations tuberculeuses et par l'issue de l'affection. En se fondant sur la "conversion" déterminée par la culture, les changements radiologiques défavorables et les taux d'oblitération des cavernes, on a estimé que plus les lésions étaient étendues moins le pronostic était favorable. Toutefois, en se fondant sur l'amélioration moyenne ou marquée des radiogrammes, on a observé un rapport inverse il y avait plus d'amélioration parmi les patients dont l'affection était plus avancée. Il est suggéré que ceci s'accorde avec le fait que l'oblitération rapide des excavations plus grandes influençait exagérément l'interprétation de l'amélioration radiologique "significative", ce qui diminue la valeur de ce critère pour le pronostic et la discrimination.

Il a été demontré que l'emploi d'une technique statistique appropriée permettait de comparer l'efficacité de différentes périodes de traitement antibactérien combiné comportant la streptomycine et le PAS, bien que cette variable n'ait pas été assignée aux patients dans la répartition au hasard. Le rapport fourni la preuve que ce traitement combiné administré pendant un minimum de douze mois est nettement supérieur à la cure de huit ou de quatre mois et qu'un traitement de dix-huit à vingt-quatre mois est légèrement, sinon significativement, supérieur à une période de traitement de douze mois.

Acknowledgments

Data for the cooperative studies reported have been contributed by the staffs of the following hospitals: Fitzsimons Army Hospital (formerly Fitsimons General Hospital), Denver, Colorado; U. S. Naval Hospitals at Sampson, N. Y.,[a] and San Diego, California (formerly located at Corona, California); and the following Veterans Administration Hospitals and Centers: Albany, N. Y.; Albuquerque, N. M.; Alexandria, La.; Aspinwall, Pa.[a]; Atlanta, Ga.; Augusta, Ga.; Baltimore, Md.; Batavia, N. Y.; Brockton, Mass.; Brecksville, Ohio; Bronx, N. Y.; Brooklyn, N. Y.; Butler, Pa.; Castle Point, N. Y.; Chamblee (Atlanta), Ga.[a]; Columbia, S. C.; Coral Gables, Fla.; Dayton, Ohio; Dearborn, Mich.; Downey, Ill.; East Orange, N. J.; Excelsior Springs, Mo.; Fort Benjamin Harrison (Indianapolis), Ind.[a]; Fort Logan (Denver), Colo.[a]; Framingham, Mass.[a]; Hines (Chicago), Ill.; Houston, Tex.; Indianapolis (Cold Spring Road), Ind.; Kerrville (formerly Legion), Tex.; Livermore, Calif.; Long Beach, Calif.; Louisville, Ky.; Madison, Wis.; Martinsburg, W. Va.; McKinney (Dallas), Tex.; Memphis (Kennedy Medical Teaching Group Hospital), Tenn.; Memphis (Lamar), Tenn.; Minneapolis, Minn.; Newington, Conn.; New Orleans, La.; Oakland, Calif.; Oteen (Asheville), N. C.; Outwood, Ky.; Portland, Ore.; Richmond, Va.; Rutland Heights, Mass.; San Fernando, Calif.; Springfield, Mo.[a]; Staten Island (Halloran), N. Y.[a]; Sunmount, N. Y.; Temple, Tex.; Tucson, Ariz.; Vancouver, Wash.; Van Nuys, Calif.[a]; Walla Walla, Wash.; Waukesha, Wis.; West Haven, Conn.; Whipple, Ariz.; and Wood (Milwaukee), Wis. It is regretted that it is impossible to list the names of the individual investigators in these hospitals who have made the studies possible.

Data compiled from forms submitted by participating hospitals have been compiled by the following statisticians in the Veterans Administration Central Office, Washington, D. C.; Morton Robins (1946–1947), Jack Beckerman (1946), Howard Dunn (1948–1949), Hyman J. Doben (1949–1951), Dorothy G. Livings (1949–1954), and John H. Williams, Jr. (1953–1954); supervision was supplied by Morton Robins, Chief, Biometrics Section (1949–1954) and Rachel M. Jenss, Assistant Chief, Medical Research Statistics Division (1949), under the following chiefs: Paul M. Densen (Chief, Medical Research Statistics Division, 1947–1949) and Daniel I. Rosen (Chief, Reports and Statistics Service, 1949–1954). Each has made a valuable contribution in the often difficult field of integration of statistical and clinical philosophies in addition to indispensable service in the handling of complex and voluminous clinical data.

From its inception the study has been guided by a perceptive "committee" in the Central Office of the Veterans Administration, at first called the Streptomycin Committee (in recent years the Committee on the Chemotherapy of Tuberculosis) under the general supervision of Dr. John B. Barnwell, Chief and Director of the Tuberculosis Service. The secretary of the committee from its beginning has been Dr. Arthur M. Walker, who has supplied constant guidance and stimulation to the project and to its individual participants.

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ISONIAZID, STREPTOMYCIN, AND PARA-AMINOSALICYLIC ACID COMPARED AS TWO-DRUG REGIMENS IN THE TREATMENT OF PULMONARY TUBERCULOSIS AMONG PREVIOUSLY UNTREATED PATIENTS[1]

III. An Account of the Cooperative Investigation of the Veterans Administration, Army, and Navy, August, 1952, to September, 1954

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(Received for publication August 9, 1955)

INTRODUCTION

Previous reports of the cooperative investigation of the effects of drug regimens in the treatment of pulmonary tuberculosis by the group of cooperating hospitals in the Veterans Administration, Army, and Navy have outlined the methodology of the study and summarized the results for the years 1946–1951, before prolonged multiple-drug therapy was being intensively investigated (1, 2). Evidence was presented in these reports that three prolonged streptomycin and para-aminosalicylic acid (PAS) regimens were therapeutically equivalent. The regimens consisted of 1 gm. of streptomycin a day, 0.5 gm. of streptomycin a day, and 1 gm. of streptomycin twice a week, along with 12 gm. of PAS daily in each instance. The period during which these three streptomycin-PAS regimens were studied was from 1951 to 1952 (3). At the Eleventh Conference on the Chemotherapy of Tuberculosis in January, 1952, investigation of the three streptomycin-PAS regimens was discontinued and, in February, 1952, a study of isoniazid was commenced. The calendar for the introduction of the different drug regimens for the study in 1952 is presented in table 1.

The investigation of the comparative efficacy of isoniazid in 1952 fell into the following segments:

February–May: A comparison of 150 mg. of isoniazid a day with a streptomycin-PAS regimen of 1 gm. of streptomycin twice weekly and 12 gm. of PAS daily

Early May to late June: A comparison of the two regimens noted above as well as a third, consisting of 300 mg. of isoniazid a day

[1] Portions of this report were presented to the Fourteenth Conference on the Chemotherapy of Tuberculosis, Atlanta, Georgia, February 7–10, 1955; and at the Medical Session, as part of *Section 1A*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 23, 1955.

[2] Prepared by the writers at the request of the Executive Committee of the Conference on Chemotherapy of Tuberculosis of the Veterans Administration, Army, and Navy.

[3] Chief, Medical Service, Veterans Administration Hospital, and Professor of Medicine, Duke University School of Medicine, Durham, North Carolina; member of Executive Committee and Chairman of Committee on Plans, Veterans Administration, Army, Navy Cooperative Study.

[4] Statistician, Reports and Statistics Service, Veterans Administration, Washington, D. C.

Late June to mid-August: A comparison of the three regimens noted immediately above in addition to a fourth, consisting of 300 mg. of isoniazid a day with 1 gm. of streptomycin twice weekly

Mid-August and subsequently: A comparison of the following three regimens:

Isoniazid-streptomycin—300 mg. of isoniazid per day plus 1 gm. of streptomycin twice weekly

Isoniazid-PAS—300 mg. of isoniazid per day plus 12 gm. of PAS daily

Streptomycin-PAS—1 gm. of streptomycin twice weekly and 12 gm. of PAS daily

Preliminary reports of the first three of these periods in 1952 have been made (4, 5). It was reported that, with relatively short-term follow-up, isoniazid alone, either in doses of 150 mg. or 300 mg. a day, was approximately as effective as

TABLE 1

CALENDAR OF DRUG REGIMENS INVESTIGATED IN 1952

Regimen*	February	May	June	August
SM-PAS	x	x	x	x
INH alone, 150 mg. per day	x	x	x	
INH alone, 300 mg. per day		x	x	
INH-SM			x	x
INH-PAS				x

* SM = streptomycin (1 gm. twice a week); PAS = para-aminosalicylic acid (12 gm. per day); INH = isoniazid (300 mg. per day except for the regimen indicated of INH alone, 150 mg. per day).

the twice-weekly-streptomycin daily-PAS regimen, except for a slightly higher relapse rate with isoniazid alone. It was further reported that either of the two drug combinations containing isoniazid (isoniazid-streptomycin or isoniazid-PAS) had approximately the same efficacy as the streptomycin-PAS regimen (5).

The present report will not concern itself further with the preliminary period of the isoniazid investigation, i.e., until August, 1952, but will consist of a presentation of the results of the last of the four periods listed above, during which three two-drug regimens have deen compared concurrently.

METHODS AND MATERIALS

The principal criterion for selection of patients for admission to the study consisted of bacteriologic confirmation of the diagnosis of tuberculosis. Other criteria, in effect in earlier periods of the study (2, 3), of a pretreatment observation period to establish the trend of the disease and of a predominantly exudative component, were no longer in effect. Patients with prior antimicrobial therapy were admitted to the study, but for the purposes of this report only those without any prior therapy or, in the presence of such therapy, only those with tubercle bacilli established *in vitro* to be susceptible to any of the drugs employed are included in this report.

Assignment of patients to the regimens under investigation was by the method of random allocation previously described (2). Certain register number final digits (three in all) were selected to apply to the isoniazid-streptomycin regimen, three to the isoniazid-PAS regimen, and four to the streptomycin-PAS regimen.

The cases of patients whose therapy was begun between August 15, 1952, when random allocation on these three regimens was commenced, and early 1954 are included in this report. Data on their progress were collected in the fall of 1954, providing a maximum eighteen-month observation period. As in the preceding report (3), results have been tabulated only on those previously untreated patients who received at least four months of antimicrobial therapy in the study.

Reversal of infectiousness, as defined for this study, means that the sputum was negative for acid-fast bacilli on microscopy and that sputum or gastric aspirates were negative for tubercle bacilli in three successive cultures obtained at a minimal interval of one month.

Other criteria employed in evaluating results, *significant roentgenographic improvement*, *unfavorable roentgenographic change*, and *cavity closure*, have been defined in the previous report (3). The identical criterion for statistical significance previously employed has been used, that of a 0.02 probability level based on a 0.99 confidence coefficient.

Status of Follow-Up Observations

In table 2 may be seen the status of follow-up of the 2,187 previously untreated patients who received at least four months of antimicrobial therapy in the study. The status is presented at eight, twelve, and eighteen months after the start of therapy in terms of the percentage under observation of those potentially under observation if all those who had received four months of therapy had been observed to the time of reporting. Expressed in such terms, the percentages under observation were 84, 59, and 37, at eight, twelve, and eighteen

TABLE 2

STATUS OF FOLLOW-UP OBSERVATIONS OF PATIENTS

Observation Status	At Observation After Start of Therapy		
	8 months	12 months	18 months
Number of patients potentially under observation*	1,891	1,427	682
	Per Cent		
Under observation, reported†	84	59	37
Reporting terminated because of:			
Change in regimen:			
Drug toxicity	1	3	3
Drug resistance	‡	1	1
Clinical decision (not toxicity or resistance)	1	4	5
Death, tuberculous	‡	‡	1
Death, nontuberculous	‡	‡	1
Irregular discharge	3	12	18
Sub-total	5	20	29
Patient discharged after course completed	1	4	10
No follow-up report submitted	9	15	18
Other miscellaneous reasons	1	2	6
Total	100	100	100

* *See* text for explanation.

† Hospitalized and nonhospitalized.

‡ Less than 0.5 per cent.

TABLE 3

DURATION OF ANTIMICROBIAL THERAPY

(Three Two-Drug Regimens)

Duration of Antimicrobial Therapy	Duration of Observation		
	8 months	12 months	18 months
Number of patients*	1,654	890	342
	Per Cent of Patients Observed		
4 months	0.5	†	†
8 months	99.5	5	5
12 months		95	17
18 months			78

* Number observed reported in this table greater than in table 2 because of technical reasons in compiling data.

† Less than 0.5 per cent.

months, respectively. As the table indicates, the largest losses from observation fell in the categories of irregular discharge (3, 12, and 18 per cent at the three observation points) and "no follow-up report submitted" (9, 15, and 18 per cent, respectively). A report that therapy was terminated for the following reasons did not necessarily mean actual loss of the patient to observation because of the technical requirement that these reasons constituted a termination of *observation for the original course of antimicrobial therapy*: change of regimen because of toxicity, drug resistance, or clinical decision; death; and "other miscellaneous reasons." In addition, many irregularly discharged patients returned to observation status.

In actuality, therefore, the losses from observation approximated 12, 20, and 30 per cent, at eight, twelve, and eighteen months, respectively. There was no significant difference in the three regimens with respect to percentage loss for any particular reason.

In table 3 are indicated the number and percentage of patients who have been observed for eight, twelve, and eighteen months. Of those observed for eight months, 99.5 per cent received at least eight months of therapy; of those observed for twelve months, 95 per cent; and, of those observed for eighteen months, 78 per cent received continuous antimicrobial therapy for eighteen months. There was no statistically significant difference in duration of therapy among the patients assigned to the three regimens.

Inter-Group Comparability

The percentages of the patients assigned to the three regimens under investigation who possessed certain defining characteristics are reported in table 4. With respect to no characteristic listed is there a statistically significant difference between any of the paired regimen comparisons. This indicates the high degree of comparability of the clinical material assigned to the three regimens resulting from the random allocation.

TABLE 4

COMPARABILITY OF PATIENTS WITH PULMONARY TUBERCULOSIS TREATED ON THREE TWO-DRUG REGIMENS BY RANDOM ALLOCATION

Defining Characteristics at Start of Therapy	Regimens*			Totals for All Three Regimens
	INH-SM	INH-PAS	SM-PAS	
Number of Patients	752	605	830	2,187
	Per Cent			
Race: Negro	15	16	18	16
Sex: male	99	99	99	99
Age: 40 years and older	52	52	52	52
Far advanced disease	48	44	49	47
Progressive pre-treatment	72	74	71	72
Bilateral disease	71	71	69	70
Multiple cavities	36	35	38	36
Cavities 4+ cm.	13	14	16	14
Prior collapse therapy or surgery	7	10	10	9

* INH = isoniazid (300 mg. per day).
SM = streptomycin (1 gm. twice weekly).
PAS = para-aminosalicylic acid (12 gm. per day).

RESULTS

In table 5 are given the results with respect to *significant roentgenographic improvement* for each of the three regimens and for all of the three added together after four, eight, and twelve months of observation. The data are likewise presented with separate groupings for patients with minimal, moderately advanced, and far advanced disease, and for lesions of all stages grouped together. In none of the inter-regimen comparisons included in the table is there a statistically significant difference in the percentage of *significant* roentgenographic improvement at any observation point for any stage of the disease or for the totals.

The data with respect to both unfavorable roentgenographic change and bacteriologic relapse for each of the three regimens, cumulatively (by the life-table procedure), up to twelve months after the start of antimicrobial therapy are summarized in table 6. In no instance is there a statistically significant difference between regimens, although it will be noted that both rates are slightly higher (unfavorable) for the patients treated on the streptomycin-PAS regimen.

In figure 1 are given the data on the reversal of infectiousness ("conversion by culture") for all patients treated on each of the three regimens, expressed in per cent of total cases observed (numbers given at the bottom of the figure) in which reversal of infectiousness had occurred at four, eight, twelve, and eighteen months after the start of therapy. Asterisks in the figure indicate that the reversal rates for the patients treated with streptomycin-PAS were significantly lower than for those treated with either isoniazid-streptomycin or isoniazid-PAS eight months after the start of therapy. The streptomycin-PAS reversal rates were also significantly lower than for those treated with isoniazid-PAS twelve months after the start of therapy.

TABLE 5

"SIGNIFICANT" ROENTGENOGRAPHIC IMPROVEMENT AMONG "ORIGINAL" TREATMENT PATIENTS TREATED WITH THREE TWO-DRUG REGIMENS BY RANDOM ALLOCATION

Regimen* and Stage of Disease	Observation After Start of Therapy					
	4 Months		8 Months		12 Months	
	Number of Patients Observed	Per Cent Improved	Number of Patients Observed	Per Cent Improved	Number of Patients Observed	Per Cent Improved
Minimal						
INH-SM	63	38	52	54	31	71
INH-PAS	47	23	35	31	11	64
SM-PAS	75	23	52	33	18	56
All 3 regimens	185	28	139	40	60	65
Moderately advanced						
INH-SM	301	49	216	61	107	72
INH-PAS	276	44	217	57	120	67
SM-PAS	314	47	229	58	112	60
All 3 regimens	891	47	662	59	339	66
Far advanced						
INH-SM	334	52	260	62	143	65
INH-PAS	254	51	182	60	108	74
SM-PAS	384	49	290	63	148	69
All 3 regimens	972	51	732	62	399	69
All Stages						
INH-SM	698	49	528	60	281	68
INH-PAS	577	46	434	56	239	70
SM-PAS	773	46	571	58	278	64
All 3 regimens	2,048	47	1,533	58	798	67

* INH = isoniazid; SM = streptomycin; PAS = para-aminosalicylic acid (for dosages see text).

In an attempt to determine what type of pulmonary tuberculosis might be most closely associated with the differences in result reflected in figure 1, the same kind of analysis was repeated according to stage of disease. The data on reversal of infectiousness for patients with minimal disease treated with the three two-drug regimens is shown in figure 2 and reveals that there was no statistically significant difference among the regimens.

In figure 3 it may be noted that eight months after the start of therapy patients with moderately advanced disease treated with streptomycin-PAS had a statistically lower rate of reversal of infectiousness than those treated with isoniazid-PAS. Twelve months after the start of therapy, the inferior showing of the streptomycin-PAS regimen, compared with the other two regimens in terms of reversal of infectiousness, was almost significant on statistical analysis.

TABLE 6

BACTERIOLOGIC RELAPSE AND UNFAVORABLE ROENTGENOGRAPHIC CHANGE (Reported Cumulatively by Life-Table Procedure to Twelve Months after Start of Therapy)

	Regimens*					
	INH-SM		INH-PAS		SM-PAS	
	Number Observed	Per Cent	Number Observed	Per Cent	Number Observed	Per Cent
A. Unfavorable roentgenographic change	315	7	263	6	312	8
B. Bacteriologic relapse	213		201		195	
Single smear positive for acid-fast bacilli		4		3		2
Single culture positive for tubercle bacilli		4		4		6
Multiple positive specimens		3		3		5
Total bacteriologic relapses		11		10		13

* INH = isoniazid; SM = streptomycin; PAS = para-aminosalicylic acid (for dosages *see* text).

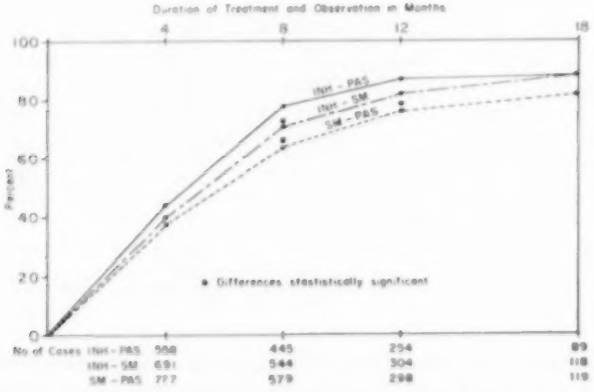

FIG. 1. Effect of regimen (second isoniazid study) on reversal of infectiousness: all cases.

The results for reversal of infectiousness for patients with far advanced disease, given in figure 4, indicate the greatest difference between regimens. Those patients treated with streptomycin-PAS had a significantly lower reversal rate than those treated with isoniazid-PAS at eight months and at twelve months after the start of therapy. Moreover, at the eight-month observation point, the difference between the lower reversal rate on streptomycin-PAS and that found for isoniazid-streptomycin closely approached significance on statistical analy-

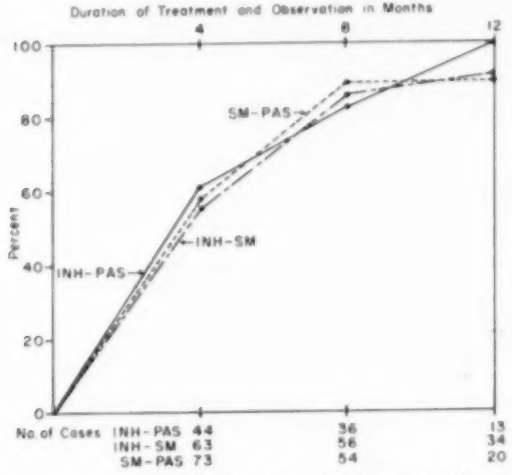

FIG. 2. Effect of regimen (second isoniazid study) on reversal of infectiousness: minimal cases.

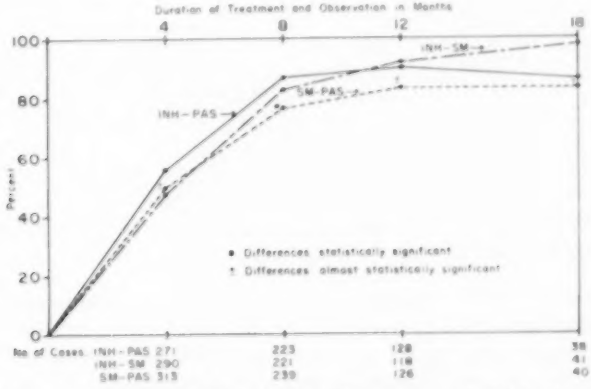

FIG. 3. Effect of regimen (second isoniazid study) on reversal of infectiousness: moderately advanced cases.

sis. In none of these three comparisons, according to stage of the disease, was there a statistically significant difference in the reversal of infectiousness between those patients treated with isoniazid-PAS and those treated with isoniazid-streptomycin. At the individual observation points, however, the results were consistently better for the group treated with isoniazid-PAS. Progressing from minimal to moderately advanced to far advanced disease (*see* figures 2 to 4), there is a progressively greater superiority of the isoniazid-PAS regimen and a progressively greater inferiority of the regimen composed of streptomycin and PAS.

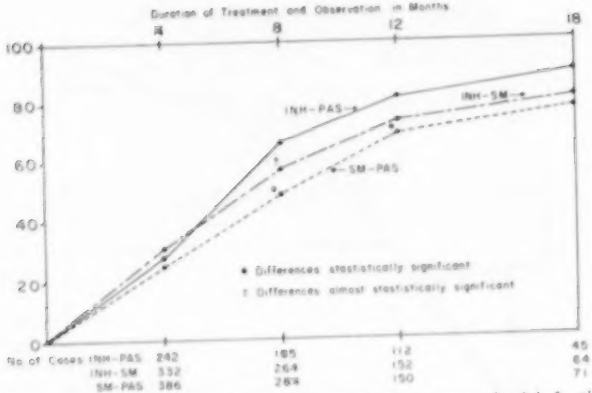

FIG. 4. Effect of regimen (second isoniazid study) on reversal of infectiousness: far advanced cases. (*"Per Cent" was inadvertently omitted from the ordinate scale.*)

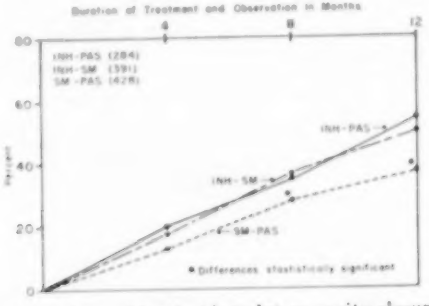

FIG. 5. Effect of regimen (second isoniazid study) on cavity closure (life table method) without resectional surgery.

Data reported with respect to cavity closure by roentgenographic observation (including tomography) were analyzed by the life-table method. They were further analyzed both for all cavitary cases (whether cavitary closure was achieved with or without resectional surgery) and separately for those patients who had not received resectional surgery prior to cavity closure. Cavity closure rates for the latter group are given in figure 5 and show that the rates for cavity closure for patients treated with streptomycin-PAS were significantly lower than for those of the other two treatment groups both at eight and at twelve months after the start of therapy. Cavity closure rates for all cavitary cases, regardless of resectional surgery, show an exactly corresponding relative relationship among the regimens. When resectional surgery was employed, the rates were higher by approximately 1, 6, and 10 per cent four, eight, and twelve months after the start of therapy, respectively.

Thus, with respect to both reversal of infectiousness and cavity closure, the results were significantly less satisfactory for those patients treated with strep-

TABLE 7

REVERSAL OF INFECTIOUSNESS ("CONVERSION BY CULTURE") AND "SIGNIFICANT" ROENTGENOGRAPHIC IMPROVEMENT RELATED TO STAGE OF DISEASE AND CAVITARY COMPONENT

Stage of Disease and Cavitary Status at Start of Therapy	Observation After Start of Treatment							
	4 Months		8 Months		12 Months		18 Months	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
A. Reversal of Infectiousness								
1. *Minimal*	180	*58*	146	*86*	67	*93*	27	*100*
2. *Moderately advanced*								
a. No visible cavities	346	*53*	269	*83*	146	*92*	41	*83*
b. Single cavities*	256	*52*	219	*84*	133	*86*	40	*95*
c. Multiple cavities*	101	*47*	73	*82*	30	*97*	14	*(100)*
3. *Far advanced*								
a. No visible cavities	124	*40*	96	*79*	62	*86*	32	*88*
b. Single cavities								
Less than 4 cm. in diameter	137	*33*	115	*62*	79	*84*	41	*85*
More than 4 cm. in diameter	75	*28*	56	*64*	35	*66*	16	*87*
c. Multiple cavities								
Less than 4 cm. in diameter†	167	*25*	130	*53*	63	*75*	14	*(100)*
More than 4 cm. in diameter†	167	*11*	113	*41*	48	*60*	9	*(67)*
B. "Significant" Roentgenographic Improvement								
1. *Minimal*	185	*28*	139	*40*	60	*65*		
2. *Moderately advanced*								
a. No visible cavities	355	*43*	264	*53*	136	*60*		
b. Single cavities*	267	*55*	210	*64*	120	*70*		
c. Multiple cavities*	102	*47*	71	*70*	28	*75*		
3. *Far advanced*								
a. No visible cavities	127	*52*	98	*67*	58	*76*		
b. Single cavities								
Less than 4 cm. in diameter	138	*57*	113	*68*	75	*72*		
More than 4 cm. in diameter	78	*71*	56	*73*	34	*76*		
c. Multiple cavities								
Less than 4 cm. in diameter†	177	*47*	131	*65*	60	*70*		
More than 4 cm. in diameter†	165	*56*	119	*69*	48	*69*		

* By definition for moderately advanced disease cavities less than 4 cm. in diameter.

† Diameter of largest cavity in multiple cavity cases.

( )Percentages in parentheses of doubtful value because of small number of cases (less than 15).

tomycin-PAS. There was some superiority (although not a statistically significant one) of the isoniazid-PAS daily regimen over the regimen of isoniazid (daily) and streptomycin (twice weekly). With respect to *significant* roentgenographic improvement or unfavorable roentgenographic change, there was no significant difference among the three regimens. With respect to *bacteriologic relapse*, there was a slight though not significant inferiority of the results attained with streptomycin-PAS.

As in the previous report (3), it has been possible to analyze the effect of variables other than those having to do with the antimicrobial therapy. The results for the three regimens have been totaled although, as the preceding presentation has indicated, there was some lack of equivalence of result among the three treatment groups. The analysis of the totals obtained in this manner, however, has been checked for each regimen separately without altering the deductions derived from the three-regimen totals.

In table 7 are presented the data on reversal of infectiousness and *significant* roentgenographic improvement for the patients treated on all three regimens according to both stage of the disease (according to *Diagnostic Standards*, 1950 edition)[5] and to its cavitary component.

With respect to reversal of infectiousness ("conversion by culture"), the following inferences may be drawn:

Minimal disease (by definition noncavernous) yielded the best results.

Moderately advanced disease, whether reported as cavernous or not, yielded approximately as good results as did minimal lesions.

Far advanced disease without detectable cavities improved almost as much as either minimal or moderately advanced disease.

In far advanced disease, the reversal of infectiousness rates for patients with single cavities, whether smaller or larger than 4 cm. in diameter, were very nearly the same although they were less in both instances than those for moderately advanced or minimal disease.

In far advanced disease there appeared to be more of an association between multiplicity of cavities than size of the largest cavity in relation to the rates of reversal of infectiousness.

The portion of table 7 reporting *significant* roentgenographic improvement reflects a quite different relative relationship according to stage of disease and cavitary component. The following may be noted:

Minimal disease exhibited the *least significant* roentgenographic improvement.

Cavernous moderately advanced disease showed more such improvement than noncavernous moderately advanced disease, and both groups exhibited more than minimal disease.

There was progressively more *significant* roentgenographic improvement among far advanced cases according to the following subgroups: noncavernous cases, cases of single cavities less than 4 cm. in diameter, and cases of single cavities 4 or more cm. in diameter. The highest rates of *significant* roentgenographic improvement were for the latter subgroup.

Rates of improvement for far advanced cases were less for patients with multiple cavities than for those with single cavities.

[5] Published by the National Tuberculosis Association.

Patients with multiple-cavitary far advanced lesions, with the largest cavity less than 4 cm. in diameter, improved less than those with the largest cavity 4 or more cm. in diameter four months after the start of therapy, but more than those with the larger cavitary component eight and twelve months after the start of therapy.

In no subgroup by stage and cavitary component was the rate for *significant* roentgenographic improvement less at any succeeding observation point than at the preceding one, except for the far advanced subgroup with multiple cavities, the largest of which was 4 cm. or more in diameter; in this subgroup, that with the most extensive disease, the improvement rate was less at twelve months than at eight months after the start of therapy.

The differences in result, according to stage and to cavitary status, between part *A* of table 7 (reversal of infectiousness) and part *B* (*significant* roentgenographic improvement) are marked and in some instances striking. For far advanced cases divided into the five subgroups indicated in the table at the four-month observation point, with respect to reversal of infectiousness, there was a progressively lower reversal rate from noncavernous cases to multiple cavitary cases with the largest cavity 4 cm. or more in diameter. With respect to *significant* roentgenographic improvement, however, the reverse is true for single-cavity and noncavernous cases. Moreover, for the noncavernous cases observed at four, eight, and twelve months, there was uniformly *less* reversal of infectiousness with the progression from minimal to moderately advanced to far advanced disease; whereas there was uniformly *more significant* roentgenographic improvement with the same progression.

Cavity closure rates have also been calculated (by the life-table method) for the patients treated on the three regimens, according to cavitary status. The results are given in figure 6 for those patients achieving cavity closure without resectional surgery, and demonstrate the following:

Cavity closure rates for the two single cavity groups (1.0 to 2.0 and 2.5 to 3.5 cm. in diameter) were approximately the same (not significantly different), and are uniformly the best.

There were progressively lower cavity closure rates for the following groups: single cavity cases (of either size), multiple cavity cases with largest cavity 1.0 to 2.0, 2.5 to 3.5, and 4.0 to 7.5 cm. in diameter.

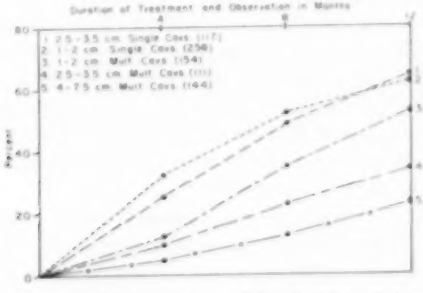

FIG. 6. Effect of cavitary component (second isoniazid study) on cavity closure (life-table method) without resectional surgery; all three regimens.

TABLE 8

REVERSAL OF INFECTIOUSNESS ACCORDING TO AGE AND RACE
Eight Months After Start of Therapy

Race	Age in Years					
	Less than 40		40-59		60 and Older	
	Number of Patients	Reversal of Infectiousness	Number of Patients	Reversal of Infectiousness	Number of Patients	Reversal of Infectiousness
		per cent		*per cent*		*per cent*
I. Moderately Advanced						
A. White	292	82	191	80	84	81
B. Negro	46	76	35	86	*	
II. Far Advanced						
A. White	208	58	232	55	96	55
B. Negro	96	54	57	56	*	

* Number of patients insufficient for analysis.

Data are also available on the effect of the variables of age and race. The data are given in table 8 with respect to reversal of infectiousness for the patients treated on all three regimens, for the patients with moderately advanced and far advanced disease considered separately, and for white and Negro patients. In addition, the data are arranged according to the following age groups: less than forty years of age, forty to fifty-nine years of age, and sixty years of age and older. In no comparison group was there a statistically significant difference in the rate of reversal of infectiousness, either with respect to race or to age group.

It is not intended in the present report to give the data in detail with respect to the rates of emergence of drug-resistant strains of tubercle bacilli. Nevertheless, as it is pertinent to the evaluation of the comparative therapeutic efficacy to consider these data, they are presented in condensed summary form in table 9. The data for this table were derived from the tabulated material presented to the Fourteenth Conference on the Chemotherapy of Tuberculosis (6) and were calculated cumulatively by the life-table method.

It is indicated in table 9 that, when either PAS daily or isoniazid daily is combined with twice-a-week streptomycin, the rates of emergence of strains of tubercle bacilli resistant *in vitro* to streptomycin are comparable for the two regimens. This is the case whether the level of resistance be defined as: growth in the 100 γ per ml. tube equivalent to that in the control tube; growth in the 100 γ per ml. tube less than that in the control tube ("partial" growth); growth in the 10 γ per ml. tube equivalent to that in the control tube; or the sum of these three degrees of *in vitro* resistance.

The data in table 9 indicate, however, that there is a definite difference in the rates at which tubercle bacilli resistant *in vitro* to 5 γ of isoniazid per ml. appear in patients treated with isoniazid-streptomycin or isoniazid-PAS. The drug-resistance rates for therapy with isoniazid (daily) and streptomycin (twice weekly) were statistically significantly higher at both eight and twelve months

TABLE 9

RATES OF EMERGENCE OF STRAINS OF TUBERCLE BACILLI RESISTANT TO STREPTOMYCIN OR ISONIAZID

(Reported Cumulatively by Life-Table Procedure to Twelve Months After Start of Therapy)

Drug Regimen*	Observation After Start of Therapy					
	4 Months		8 Months		12 Months	
	Number of Patients Observed	Per Cent with Resistant Strains	Number of Patients Observed	Per Cent with Resistant Strains	Number of Patients Observed	Per Cent with Resistant Strains
A. Streptomycin Resistance						
SM-INH	568		500		287	
SM-PAS	636		535		275	
Equivalent growth in 100 γ per ml.						
SM-INH		1		1.5		3
SM-PAS		1		2		3
Partial growth in 100 γ per ml.						
SM-INH		3		4.5		4.5
SM-PAS		2		4		5
Equivalent growth in 10 γ per ml.						
SM-INH		1		2		4.5
SM-PAS		2.5		2		2
All levels of resistance						
SM-INH		5		8		12
SM-PAS		5.5		8		10
B. Isoniazid Resistance						
Equivalent growth in 5 γ per ml.						
INH-SM	575	3	501	5	288	7
INH-PAS	464	1	415	1.5	239	2

* INH = isoniazid; SM = streptomycin; PAS = para aminosalicylic acid (for dosages *see* text).

after the start of therapy and were almost significantly higher at four months than for isoniazid-PAS administered daily. A more detailed presentation of drug-resistance and susceptibility data from the cooperative investigation is planned for a subsequent report.

DISCUSSION

The primary purpose of the investigation here reported has been to determine the relative efficacy on concurrent study of two two-drug regimens containing isoniazid (isoniazid, 300 mg. daily, plus 1 gm. of streptomycin twice weekly, and isoniazid, 300 mg. daily, plus 12 gm. of PAS daily) and a streptomycin-PAS regimen (1 gm. of streptomycin twice weekly plus 12 gm. of PAS daily).

In studies such as the present investigation, it is essential that it be satisfactorily established that the treatment groups are comparable. In the present study, the principle of random allocation of the three regimens to patients has been employed meticulously and evidence has been presented (*see* table 4) that the three groups are in fact highly comparable. Evidence was further presented that the interpretation of the results concerning possible differences among the regimens might be safely attributed to the therapeutic variables deliberately introduced into the study rather than to possible differences in the clinical material. Further refinements in methodology, for example, the insistence that "conversion rates" be based on reversal of infectiousness of the patient for at least a two-month period, have to an additional degree made the observations reported more objective and less subject to the vagaries of interpretation by the individual cooperating investigators.

A considerable proportion of this presentation has dealt with the apparent inconsistency in results according to the observation *significant* roentgenographic improvement and according to such other observations as reversal of infectiousness and cavity closure. The inconsistency noted in table 7 between the results according to *significant* roentgenographic improvement and reversal of infectiousness according to stage of disease and cavitary status is particularly striking. Analysis of data from the 1951–1952 study of three streptomycin-PAS regimens showed a similar inconsistency (3). It is possible that these inconsistencies are related to the interpretation of roentgenographic change by the individual investigators rather than by disinterested readers unaware of the course and nature of the treatment of the patients. The latter procedure has been followed by the United States Public Health Service cooperative group (7, 8) and the British Medical Research Council (9). It does not seem likely, however, that the inter-category inconsistencies noted in the present study are a reflection of the particular method used. In support of this position is the fact that these disparities among the bacteriologic and the two kinds of roentgenographic findings were found among the Veterans Administration, Army, and Navy group from hospital to hospital and from study to study. Moreover, a test of the roentgenographic interpretations of the present study, in which readings by investigators and by an independent "jury" of experts were compared, showed that there was an adequate comparability of the results by either method (10). It is concluded, therefore, that, with the present system of interpreting serial roentgenograms, the finding of *significant* roentgenographic improvement has less reliability than other more objective measures. Muschenheim has recently said "When one comes to attaching great significance to 1-plus, 2-plus, or 3-plus 'improvement' and attempts a determination of the 'best' treatment on statistical analysis of such arbitrary data, one is building on a somewhat unstable foundation" (11).

This is not to say that such serial roentgenographic observation has no merit in the evaluation of antimicrobial therapy, but rather stresses the obvious, that it is a relatively unreliable tool for precise evaluation of the results of such therapy. In view of this conclusion, therefore, interpretations of differential effects

related to any variable of the tuberculous disease or to its therapy which place reliance upon roentgenographic change should at least be confirmed by other observations before any final conclusions are drawn.

In this report, as in the previous two reports which cover studies by the group of cooperating hospitals in the Veterans Administration, Army, and Navy from 1949–1952, very little mention has been made of the important finding of the respective drug susceptibilities of the strains of tubercle bacilli recovered from the patient considered in relation to the drugs employed in the test regimens. This does not reflect any belief that such findings are not important either to the patient or to the interpretation of results; it is merely postponing the more complete evaluation until such time as these data can be adequately presented. In the previous study, in which three streptomycin-PAS regimens were compared (3), the preliminary analyses actually showed essential comparability of the rates of emergence of streptomycin- or PAS-resistant strains of tubercle bacilli for the three regimens. Accordingly, the postponement of a complete presentation of the drug-susceptibility data is not believed to have altered the validity of the interpretations made. In the present study, a brief presentation has been made of the comparative rates of emergence of streptomycin-resistant and isoniazid-resistant strains of tubercle bacilli, and these data will be discussed further below.

Although the cooperative group of hospitals in the Veterans Administration, Army, and Navy has conducted trials of the relative efficacy of isoniazid alone, the present report does not cover the period from February to August, 1952, when these trials were in effect. In view of opinions published recently to the effect that isoniazid alone, at least for certain types of tuberculous conditions, may be as effective as isoniazid-streptomycin or isoniazid-PAS (11, 12, 13), and that the higher rate of emergence of isoniazid resistance with isoniazid alone may not necessarily be related to a more unfavorable course of the disease (14), review of the data obtained in early 1952 is of considerable importance and is, in fact, in progress at the present time, with more extensive follow-up than was earlier possible.

As Muschenheim has pointed out (11), ". . . it seems highly probable that the best management of tuberculosis will prove to be, not a single drug regimen which is optimal for all cases . . . but that *different* regimens will be found to best suit *different* forms and types of the disease." Accordingly, it has been of interest in the present study to examine the relative efficacy of the three regimens investigated according to a number of variables of the disease itself.

It was found, for example, that the three two-drug regimens are approximately equally effective for minimal pulmonary tuberculosis, but that the isoniazid-PAS daily regimen is more effective for the more advanced cases and for those with the largest cavitary components. It was further found that the regimen of streptomycin twice weekly and PAS daily was definitely less effective for these last two groups.

The first isoniazid study of the United States Public Health Service reported that the rates for *significant* roentgenographic improvement were the same for

minimal, moderately advanced, and far advanced cases of pulmonary tuberculosis treated with isoniazid, isoniazid-streptomycin, or streptomycin-PAS (7*f*). These findings are in contrast to the findings of the present study, in which progressively more such improvement was noted the more advanced the stage of the disease. In contrasting these findings, it is necessary to note certain differences in the two investigations. The Public Health Service study included a certain percentage of patients who had had prior antimicrobial therapy (although all were *streptomycin-susceptible*); it included 60 per cent of females; and it employed a different method for roentgenographic interpretation. The Veterans Administration-Army-Navy study included only previously untreated patients and contained less than 1 per cent of females. It is not altogether clear whether these differences account for the observed differences in *significant* roentgenographic improvement.

The Public Health Service study also found a relationship between the age of the patient and *significant* roentgenographic improvement; the older the patient group, the less favorable the response (7*f*). In the Veterans Administration-Army-Navy investigation, the effect of age groups has also been tested. For patients treated with antimicrobial therapy of relatively short duration, reported in the first article of this series (2), it was found that patients more than forty-five had significantly more roentgenographic improvement, designated as moderate or marked, than those less than forty-five (15). For patients treated with longer courses of antimicrobial therapy reported in the second paper of the series (3), there was definitely more *significant* roentgenographic improvement in the group *less than forty years of age* than in that more than forty years of age (16). In the present series there is *no* significant difference in reversal of infectiousness rates for age groups less than forty, forty to fifty-nine, and sixty years of age and older (table 8), according to race or stage of disease. In view of these conflicting findings, but especially in the light of the data presented in table 8, it is probable that the factor of age per se does not influence the outcome of antimicrobial therapy as much as do other associated factors. As the Public Health Service studies (7*f*) and Reisner and associates (13) have pointed out, disease of recent origin responds most favorably, and it is altogether likely that this factor rather than age is the important variable in such correlations.

The Public Health Service investigators found, with respect to the influence of race, that there was less difference with respect to *significant* roentgenographic improvement between whites and non-whites than between age groups. "In those under 45 years old the proportion who improved was greater among whites than non-whites, while in those over 45 years old more non-whites than whites showed X-ray film improvement" (7*f*). The present report indicates no significant difference with respect to reversal of infectiousness between whites and non-whites for either moderately advanced or far advanced disease groups, or for different age groups (table 8). Thus, in spite of differences in the two studies, there is approximate agreement that with effective antimicrobial therapy of the type employed the factor of race per se probably has little influence on the outcome.

The previous report in this series has indicated that the results are less satisfactory with bilateral involvement than with unilateral (3); the Public Health Service data are in agreement with those findings (*7f*).

It has not been possible in the present investigation to analyze the results according to duration of the antimicrobial therapy. As indicated in table 3, there were practically no patients treated for less than eight months, and only 5 per cent of the total who were observed to twelve months were treated for eight months. It is possible, however, that, as further data are reported on patients in this study,[8] it may be practicable to extend the analysis begun with patients treated with streptomycin-PAS (3) and to determine the relative value of courses of antimicrobial therapy of twelve, eighteen, and twenty-four months' duration in patients who have received no previous chemotherapy.

The principal findings of the investigation of regimens employing isoniazid, streptomycin, and PAS in three two-drug combinations are three in number: isoniazid-PAS administered daily consistently gives the best results; the results from this regimen are slightly superior to those from the regimen of isoniazid daily and streptomycin twice weekly; the results with the regimen combining streptomycin twice weekly and PAS daily are the least satisfactory, especially in the most advanced cases and in those with the largest cavitary component.

It is interesting to compare the results of the cooperative investigations of the Veterans Administration, Army, and Navy (VAAN) with those of the British Medical Research Council (MRC) (9) and the second isoniazid study of the United States Public Health Service (PHS) (8). All three employed rigid criteria for admission to the study, including bacteriologic proof; the MRC trials admitted patients with prior antimicrobial therapy but only in very small amounts (less than 15 gm. of streptomycin, 300 gm. of PAS, or 3 gm. of isoniazid); patients admitted to the other two studies were all previously untreated. The duration of therapy in the MRC trials was for three months, with freedom of therapy thereafter, but a considerable number received the original therapy for at least six months. The duration of therapy in the PHS study was for forty weeks, the results available to thirty-two weeks being reported. In the VAAN study, the duration of therapy was, in general, for more than twelve months. All three studies employed the principle of random allocation of regimens to patients under investigation although different techniques were followed. In all three studies, patient-treatment groups resulting from such random allocation were found to be highly comparable in their composition.

There were definite differences in the drug regimens investigated, as follows:

The MRC trials compared:

1. streptomycin daily and PAS (20 gm. per day) daily
2. streptomycin twice weekly plus PAS daily
3. isoniazid (200 mg. per day) daily plus 20 gm. of PAS daily
4. isoniazid (200 mg. per day) daily plus 10 gm. of PAS daily

[8] For the Fifteenth Conference on the Chemotherapy of Tuberculosis, to be held in February, 1956.

The PHS study compared five regimens employing isoniazid in various combinations:

1. 3 mg. of isoniazid per kg. daily plus streptomycin twice weekly
2. 10 mg. of isoniazid per kg. daily plus streptomycin twice weekly
3. 3 mg. of isoniazid per kg. daily plus PAS (10 to 12 gm. per day) daily
4. 10 mg. of isoniazid per kg. daily plus PAS daily
5. 10 mg. of isoniazid per kg. daily plus streptomycin twice weekly and PAS daily.

The three regimens studied by the VAAN group were:

1. isoniazid (300 mg. per day) daily plus streptomycin twice weekly
2. isoniazid daily plus PAS (12 gm. per day) daily
3. streptomycin twice weekly plus PAS daily

If certain noncomparable regimens are excluded from these several regimens, the experience of the three cooperative groups may be compared, keeping in mind the differences in methodology and clinical material noted.

Two regimens, with very minor differences, are common to all three studies: (*A*) isoniazid daily and streptomycin, 1 gm. twice weekly (MRC—isoniazid dosage: 200 mg. per day; PHS—3 mg. per kg. per day = 150 to 250 mg. per day; VAAN—300 mg. per day); and (*B*) isoniazid daily and PAS daily (MRC—20 gm. of NaPAS = 15 gm. PAS per day; PHS—10 to 12 gm. per day; VAAN—12 gm. per day). A third regimen (*C*) studied by the MRC will also be referred to, consisting of *isoniazid and streptomycin daily*.

Results from the MRC trials indicated that regimen *B* (isoniazid-PAS daily) was superior to regimen *A* (isoniazid daily and streptomycin twice weekly) (9*g*). The PHS study concluded that regimen *B* is not clearly superior to regimen *A*, but data from this study show a consistent superiority for regimen *B* over regimen *A*, with respect to reversal of infectiousness if not for *significant* roentgenographic improvement (8*b*, *c*). The VAAN study has indicated a slight but consistent advantage for regimen *B* over regimen *A* based on roentgenographic and bacteriologic criteria.

The MRC trials have been the only ones to compare isoniazid daily plus streptomycin daily (regimen *C*) with isoniazid daily plus streptomycin twice weekly (regimen *A*); their results demonstrate clearly the superiority of the former, especially when drug resistance is taken into account (9*g*). Cohen has proposed the hypothesis, supported by a substantial amount of data from the PHS study, that "the question 'which patients will have drug-resistant bacilli?' is really the same question as 'which patients will continue to produce "positive" sputum?' " (17). All three studies show a high degree of loss of susceptibility to isoniazid of the strains of tubercle bacilli from those patients who continued to discharge tubercle bacilli after five to six or more months of therapy.

The data for isoniazid resistance presently available from the three studies are summarized in table 10. Resistance *in vitro* to 1 to 5 γ of isoniazid per ml. with therapy consisting of isoniazid-PAS daily appears to be consistent in all three studies, ranging from 0 to 2 per cent of all patients tested four to eight months after the start of therapy. The rate for therapy consisting of isoniazid daily

TABLE 10

RATES OF EMERGENCE OF STRAINS OF TUBERCLE BACILLI RESISTANT TO ISONIAZID FROM THREE COOPERATIVE STUDIES

Time of Observation	British Medical Research Council (*reference 9g*) 4–6 months	U.S. Public Health Service (*reference 8c*) 24 weeks	U. S. Veterans Administration Army and Navy (*table 9*) 4–8 months
Regimen*	Per Cent of Patients Observed with Organisms Resistant to		
	1–5 γ/ml.	1–5 γ/ml.	5 γ/ml.
A. INH-SM (twice weekly)	8–13	13	3–7
B. INH-PAS	0–2	2	1–2
C. INH-SM (daily)	0–2	—	—

* INH = isoniazid; SM = streptomycin; PAS = para-aminosalicylic acid.

plus streptomycin twice weekly is in close agreement in the MRC and PHS studies; that it is lower in the VAAN study probably is related to the fact that its isoniazid data are for resistance to 5 γ per ml. In all three studies the differences in percentages between these two regimens are statistically significant. The MRC report indicates the same incidence of isoniazid resistance for the regimen of isoniazid and streptomycin daily as for that of isoniazid and PAS daily.

It is clear from the present study and the other studies cited that the differences in the results among the various regimens are not great but that, in most of the more advanced types of pulmonary tuberculosis, the results are superior with a regimen employing isoniazid as one of a multiple-drug combination. The present study has not included a comparison of so-called "triple-drug therapy," isoniazid-streptomycin-PAS. Preliminary results from a pilot study reported to the Fourteenth Conference on Chemotherapy by the VAAN group in February, 1955, however, did not reveal a clear superiority of the three-drug regimen over either isoniazid-streptomycin or streptomycin-PAS (18). Moreover, the second PHS isoniazid study has not found such "triple-drug therapy" to be "more effective than the combination of either streptomycin or PAS with isoniazid" (8c).

* * *

Thus, within approximately three years after isoniazid began to be investigated extensively in clinical trials, it has been firmly established that the antimicrobial therapy of choice for tuberculosis should include isoniazid. The issue whether isoniazid alone may be employed under certain circumstances is not altogether clear, as has been indicated, but it is probable that there is a definite place for such therapy when the disease is not advanced. The choice of the second drug to use along with isoniazid when multiple-drug therapy is advisable is even less clear and must be decided with due regard to factors other than those which have been employed in the studies here reported.

Judging from the evidence presently available, it is probable that the regimen of isoniazid daily and streptomycin daily is that which leads consistently to the

best results, as indicated by the MRC trials (9). The two-drug regimen of isoniazid daily and streptomycin twice weekly leads to results almost as good as those with daily streptomycin, as judged by the clinical observations listed, but has the definite disadvantage of being associated with a higher rate of emergence of isoniazid-resistant strains of tubercle bacilli. On the other hand, the regimen of isoniazid-PAS administered daily has been found to be very effective. The MRC study concludes with respect to this regimen that it "has proved itself a very effective combination of drugs, although not quite so powerful as daily streptomycin plus isoniazid" (9*g*). Furthermore, both the MRC trials (9*g*) and the PHS studies (8*c*) have shown that smaller doses of PAS than those customarily administered (10 gm. a day of the sodium salt in the MRC trials, 5 to 6 gm. of PAS daily in the PHS study) when administered with isoniazid are apparently as effective as the larger usual doses and are less toxic.

The PHS investigators have stated that "The choice between streptomycin and PAS as partners for isoniazid must be made on other grounds than therapeutic effectiveness" (8*b*). Muschenheim has pointed out that "There is little indication . . . which is the preferable drug, streptomycin or PAS, to use as a companion to isoniazid"; and that "There are some theoretical objections, at least, to the streptomycin-isoniazid combination, because it commits the two most effective agents simultaneously." He believes these objections are more theoretical than practical, especially in view of the high rate of reversal of infectiousness with the regimen and the availability of other drugs when drug resistance does occur (11).

The cooperative studies of the antimicrobial therapy of pulmonary tuberculosis have not answered with final precision, therefore, the question which regimen is "best," but have laid the basis with factual information for the practical clinical decision to be made by the well-informed physician.

SUMMARY

Isoniazid, streptomycin, and para-aminosalicylic acid (PAS) have been compared as two-drug regimens in the treatment of bacteriologically proved pulmonary tuberculosis. A total of 2,187 patients without prior drug therapy were assigned by random allocation one of the following three regimens:

Isoniazid, 300 mg. per day, plus 12 gm. of PAS per day
Isoniazid, 300 mg. per day, plus 1 gm. of streptomycin twice weekly
Streptomycin, 1 gm. twice weekly, plus 12 gm. of PAS per day

Analysis of factors characterizing the patients and their disease showed the resulting three treatment groups to be highly comparable.

The best results, with respect to the criteria reversal of infectiousness ("conversion by culture"), unfavorable roentgenographic change, and cavity closure, were obtained with the isoniazid-PAS regimen; the poorest, with streptomycin-PAS. The results with isoniazid daily plus streptomycin twice weekly were slightly less good than those with isoniazid-PAS administered daily. The more advanced the disease and the greater the cavitary component, the more marked was this differentiation.

Analysis of the results according to the criterion *significant* roentgenographic improvement (moderate plus marked degrees of improvement) showed it to be poorly correlated with the other criteria listed, especially with respect to the stage of the disease and the cavitary component of more advanced disease. It is concluded, therefore, that the criterion *significant* roentgenographic improvement is of restricted value in such studies and that greater reliance should be placed on other more objective measures.

Analyzing the influence of variables other than those of the antimicrobial drugs employed, evidence has been presented which indicates that race and age per se do not appear to be clearly associated with outcome; that results are less satisfactory the more advanced the disease and the larger its cavitary component at the start of therapy; and that bilateral involvement responds less well than unilateral. It has not been possible in the investigation here reported to analyze the relative influence of differing durations of therapy. The vast majority of patients were treated for more than twelve months, but the evidence continues to suggest that twelve months of therapy is the minimum advisable and that more may be advisable in most patients.

Data have been presented which indicate that, when either isoniazid or PAS is administered daily as the companion to streptomycin twice weekly, rates for the emergence of streptomycin-resistant strains of tubercle bacilli are comparable. When PAS daily is compared with streptomycin twice weekly as the companion for daily isoniazid, however, the rates for the emergence of drug-resistant organisms (resistant *in vitro* to isoniazid concentrations of 5 γ per ml.) are significantly higher for the daily isoniazid and twice-weekly streptomycin regimen. Reference has been made to the fact that the clinical significance of this finding, however, is not clearly established.

Comparisons have been made between the results of the cooperative investigations of the Veterans Administration, Army, and Navy, of the United States Public Health Service group, and of the British Medical Research Council trials. There is good agreement that regimens which employ isoniazid with either streptomycin or PAS are approximately equally effective, but that streptomycin twice weekly as the companion for isoniazid is slightly less effective than isoniazid-PAS. The Medical Research Council trials have indicated that the combination of daily isoniazid and daily streptomycin gives consistently the best results.

There is increasing evidence that the final decision as to what antimicrobial therapy will be prescribed in practical clinical application will depend on variations of the disease and on other factors not included in the large-scale British and United States cooperative studies.

SUMARIO

La Isoniacida, la Estreptomicina y el Acido Para Aminosalicílico Comparados como Regímenes Bi-Medicamentosos en el Tratamiento de la Tuberculosis Pulmonar Entre Enfermos No Tratados Antes. III. Reseña de la Investigación Cooperativa Llevada a Cabo por la Administración de Veteranos, el Ejército y la Armada, agosto, 1952, a septiembre, 1954

La isoniacida, la estreptomicina y el ácido para aminosalicílico (PAS) fueron comparados en calidad de regímenes de dos drogas en el tratamiento de la tuberculosis pulmonar comprobada bacteriológicamente. A un total de 2,187 enfermos sin farmacoterapia anterior se les asignó al azar uno de los siguientes tres regímenes:

Isoniacida, 300 mgs. al día, más 12 gms. de PAS al día.

Isoniacida, 300 mgs. al día, más 1 gm. de estreptomicina dos veces semanales.

Estreptomicina, 1 gm. dos veces semanales, más 12 gms. de PAS al día.

El análisis de los factores que caracterizaban a los enfermos y a su dolencia demostró que los tres grupos terapéuticos eran sumamente comparables.

Los mejores resultados, con respecto a las pautas de inversión de la infecciosidad ("viraje de los cultivos"), desfavorables alteraciones radiográficas y cierre de cavernas, se obtuvieron con el régimen de isoniacida-PAS, los peores, con la estreptomicina-PAS. Los resultados con la isoniacida diaria más estreptomicina bisemanal fueron un poco menos buenos que los conseguidos con la isoniacida-PAS administrados diariamente. Mientras más avanzada la enfermedad y mayor el componente cavitario, más notable era esta diferenciación.

El análisis de los resultados de acuerdo con la pauta de *importante* mejoría radiografía (mejoría de grado moderado más o acentuado) reveló que se correlacionaba mal con las otras pautas enumeradas, y sobre todo con el período de la envermedad y el componente cavitario de la enfermedad más avanzada. Dedúcese, por lo tanto, que la pauta de *importante* mejoría radiográfica posee valor limitado en tales estudios, debiéndose depositar mayor fe en otras medidas más objetivas.

Analizando el influjo de factores variables, distintos de los correspondientes a las drogas antimicrobianas empleadas, preséntanse datos indicativos de que la raza y la edad en sí mismas no parecen asociarse netamente con el desenlace; que los resultados son menos satisfactorios mientras más avanzada está la enfermedad y mayor es el componente cavitario al iniciarse la terapéutica; y que la invasión bilateral responde menos bien que la unilateral. En la investigación aquí descrita, no ha sido posible analizar el relativo influjo de distintas duraciones de la terapéutica. La inmensa mayoría de los enfermos fué tratada más de doce meses, pero los datos acopiados continúan sugiriendo que doce meses de terapéutica es el mínimo conveniente y que en la mayor parte de los enfermos tal vez convenga aun más.

Preséntanse datos indicativos de que, cuando se administra diariamente ya isoniacida o PAS como compañero de la estreptomicina bisemanalmente, son comparables los coeficientes de aparición de cepas estreptomicinorresistentes de bacilos tuberculosos. Sin embargo, cuando se compara el PAS diariamente con la estreptomicina bisemanalmente como compañero de la isoniacida diariamente, los coeficientes de aparición de microbios farmacorresistentes (resistentes *in vitro* a una concentración de 5 γ por ml. de isoniacida) son significativamente más altos para el régimen de isoniacida diaria y estreptomicina bisemanal. No obstante, menciónase el punto de que no se ha establecido definitivamente la importancia clínica de este hallazgo.

Se han trazado comparaciones entre los resultados de las investigaciones cooperativas de la Administración de Veteranos, del Ejército y de la Armada, del grupo del Servicio de Sanidad Pública de los Estados Unidos y de los ensayos del Consejo Británico de Investigación Médica. Existe buen acuerdo acerca de que los regímenes que emplean isoniacida ya con estreptomicina o con PAS son aproximadamente de igual eficacia, pero que la estreptomicina bisemanal como compañera de la isoniacida es ligeramente menos eficaz que la isoniacida-PAS. Los ensayos del Consejo de Investigación Médica han indicado que la combinación de isoniacida a diario y estreptomicina a diario da constantemente los mejores resultados.

Van en aumento las pruebas de que el fallo definitivo en cuanto a qué terapéutica antimicrobiana será prescrita en la aplicación clínica práctica se basará en las variaciones de la dolencia y en otros factores que no figuran en los estudios cooperativos en gran escala de Inglaterra y los Estados Unidos.

RESUME

Isoniazide, Streptomycine, Acide Para-Aminosalicylique: Etude comparative des résultats obtenus par l'association de deux antibiotiques dans le traitement de la tuberculose pulmonaire chez les malades non traités antérieurement. III. Compte-rendu de l'investigation collective de l'Administration des Vétérans, Armée et Marine, Août 1952-Septembre 1954

Etude comparative des résultats obtenus avec l'isoniazide, la streptomycine et l'acide para-aminosalicylique par l'association de deux de ces antibiotiques dans le traitement de la tuberculose pulmonaire confirmée bactériologiquement. Un nombre total de 2 187 patients, n'ayant jamais été traités par les antibiotiques, ont été répartis au hasard dans les groupes traités par les cures suivantes:

Isoniazide, 300 mg par jour, plus PAS, 12 mg par jour
Isoniazide, 300 mg par jour, plus streptomycine, 1 g deux fois par semaine
Streptomycine, 1 g deux fois par semaine, plus PAS 12 g par jour

L'analyse des facteurs spécifiques relatifs aux malades et à leur maladie a montré que les trois groupes étaient nettement comparables.

Les meilleurs résultats, à l'égard des critères suivants: négativation des crachats ("conversion" en culture), modification défavorable des signes radiologiques et oblitération des cavernes furent obtenus avec la cure isoniazide PAS; les moins bons avec la cure streptomycine-PAS. Les résultats obtenus avec l'isoniazide quotidienne plus la streptomycine bi-hebdomadaire furent un peu moins bons que ceux du traitement isoniazide-PAS quotidien. Plus la maladie était avancée et le facteur excavation marqué, plus cette différenciation était accentuée.

L'analyse des résultat selon le critère amélioration *significative* des radiogrammes (c'est à dire de moyenne à marquée) a montré que la corrélation entre ce critère et les autres était médiocre, surtout à l'égard du stade de la maladie et du facteur excavation pour la maladie au stade plus avancé. En conclusion, le critère de l'amélioration radiologique "*significative*" est d'une valeur restreinte dans ces études et il y a lieu de se fier à des déterminations plus objectives.

Par l'analyse de l'influence des variables autres que celle des agents antibactériens utilisés, les auteurs présentent des données qui indiquent que la race et l'âge, en soi, n'apparaissent pas clairement associés au pronostic; que les résultats sont d'autant moins satisfaisants que la maladie est plus avancée et les excavations plus grandes au début du traitement; que l'atteinte bilatérale répond moins bien au traitement que l'affection unilatérale. Dans l'investigation rapportée, il n'a pas été possible d'analyser l'influence relative de la durée des cures. Les malades ont été en grande majorité traités pendant plus de douze mois, mais les données que l'on possède montrent qu'un traitement de douze mois représente le minimum recommandable, et qu'il y aurait intérêt à en prolonger la durée chez la plupart des malades.

L'auteur présente des données qui indiquent que lorsque l'isoniazide ou le PAS sont administrés quotidiennement avec association de streptomycine deux fois par semaine, les taux d'apparition de souches de BK streptomycino-résistantes sont comparables. Cependant, quand le PAS quotidien est comparé à la streptomycine bi-hebdomadaire comme agent associé à l'isoniazide quotidien, les taux d'apparition des germes résistants (résistant *in vitro* à l'isoniazide à la contration de 5 γ par ml.) sont significativement plus élevés avec le traitement par l'isoniazide quotidien associée à la streptomycine bi-hebdomadaire. L'attention est attirée sur le fait que la signification clinique de ce phénomène n'est pas nettement établie.

Une étude comparative des résultats de l'investigation collective de la Veterans Administration, Army and Navy, du groupe United States Public Health Service et de l'expérimentation du British Medical Research Council a été effectuée. Les cures qui comportent l'isoniazide avec association de streptomycine ou de PAS sont reconnues comme ayant sensiblement la même efficacité, tout en considérant que la streptomycine bi-hebdo-

maladie associée à l'isoniazide est légèrement moins efficace que l'isoniazide-PAS. L'expérimentation du Medical Research Council a indiqué que l'association de l'isoniazide quotidien et de la streptomycine quotidienne donnait régulièrement les meilleurs résultats.

Il de produit une accumulation de données démontrant que le jugement définitif, sur ce que sera la thérapie antibactérienne appliquée en clinique, dépendra des variations de la maladie et d'autres facteurs qui n'ont pas été inclus dans les études collectives à grande échelle effectuées aux Etats-Unis et an Angleterre.

Acknowledgments

The list of hospitals participating in this study was given in the first and second reports of this series (2, 3), as well as the names of the individuals in the Reports and Statistics Service of the Veterans Administration who have continued to carry the burden of assembling and presenting statistical data. Hundreds of individuals in the participating hospitals have contributed their data anonymously. The program has remained under the efficient direction of the Committee on the Chemotherapy of Tuberculosis of the Veterans Administration, Dr. Arthur M. Walker, Secretary.

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MAXIMAL MIDEXPIRATORY FLOW[1, 2, 3]

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INTRODUCTION

Detection and description of ventilatory abnormalities can be effected by means of a testing procedure which consists of performing a maximally rapid and deep expiration. Early users of this procedure included Baraeh (1), Gross (2), and Tiffeneau and associates (3, 4). Numerous writers (5–14) have agreed on the clinical utility of the test but have not agreed on the method by which the spirometric recording should be measured. Moreover, the mechanical limitations of the recording spirometers have not been widely recognized (15).

The present report is concerned with the results obtained in a study of healthy persons and patients who had pulmonary disease, in whom both pneumotachographic and spirometric recordings were made. These data augment the normal standards for certain measurements used previously. A new measurement, namely that of maximal midexpiratory flow, is proposed as a simple and more sensitive means for the detection of expiratory obstruction.

OBSERVATIONS

Flow Patterns of Maximally Rapid and Deep Expiration: Preliminary Studies

Patterns of flow were measured with a pneumotachograph similar to that of Lilly (16). Its resistance to the flow of air was 2 mm. of water per 100 liters per minute. The pressure transducer used was a differential transformer-amplifier system similar to that described by Ellis and associates (17). Recording was made by means of an oscillograph galvanometer (with a natural frequency of 40 cycles per second) and a photokymograph with a paper speed of 75 mm. per second. One hundred per cent response to an approximate square wave of flow of 0 to 250 liters per minute was achieved in 0.04 second. This was sufficiently rapid for the purposes of the study. Only negligible differences were observed when simultaneous recordings of forced expirations were made with this pneumotachograph and with another which used a differential strain-gauge manometer (Statham P 97) coupled to an oscillograph galvanometer and gave 100 per cent response in 0.015 second.

Patterns in health and in emphysema: A total of 25 recordings was made in the sitting position from 6 healthy men and 3 healthy women. The subjects, after a maximal inspiration, expired through a mouthpiece and 75 cm. of smooth-bore plastic tube having an inner diameter of 2.5 cm. and attached to the flowmeter. A typical pattern of flow is shown in figure 1. Initially, for approximately 0.1 second, a rapid increase of flow took place, reaching peak rates of 8 to 11 liters

[1] From the Mayo Foundation (of the University of Minnesota) and the Mayo Clinic, Rochester, Minnesota.

[2] Presented in part before the Medical Session, as part of *Section 2A*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 23, 1955.

[3] A portion of this material is included in the thesis submitted by Dr. Leuallen to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

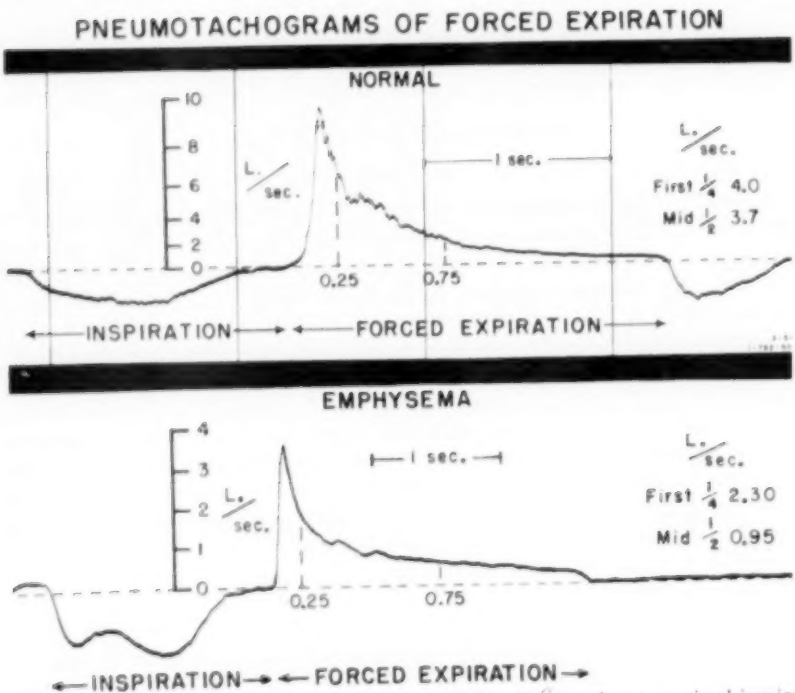
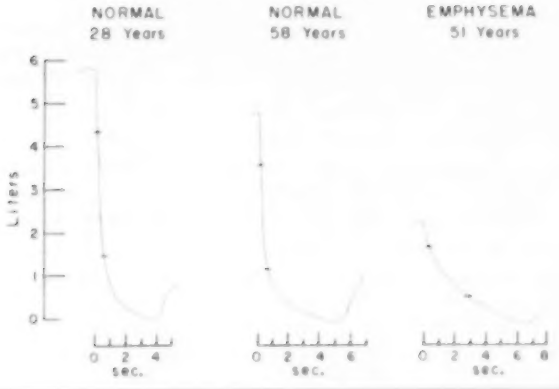


FIG. 1. Pneumotachograms obtained during forced expiration after a maximal inspiration. Upper panel shows typical pattern in a normal person, whereas the lower panel shows the pattern in a patient who had emphysema.

per second in the men and 5 to 7 liters per second in the women. The initial deflection in many records was more abrupt than that shown in figure 1, approaching a right angle in some instances. After peak flow, the rate of flow progressively decreased, although numerous small irregularities were present.

Records also were obtained from 10 men who had pulmonary emphysema. As illustrated in figure 1, these records also showed a brief period of increasing flow, with peak rates of 1 to 3 liters per second, followed by a rapid decrease to the lower rates of flow prevailing throughout the remainder of the expiration. Large accelerations and decelerations of volume occurred in both normal persons and patients who had emphysema during the first several tenths of a second. This indicated that the inertial factors present in the usual type of water-sealed cylindric recording spirometer would render it unsuitable for faithful recording. During the middle and later parts of the expiration, however, the changes in rate of flow were less rapid and more suitable for accurate recording by a spirometer.

The pneumotachograms also confirmed certain gross characteristics of the pattern of expiratory flow of patients who have emphysema which have been



Volume (L.)	5.8	4.8	2.4
% Expired			
in 1 second	84	81	46
in 3 seconds	99	98	77
Flow Rate of Mid-Half (L./sec.)	5.7	4.3	0.5
Duration of Mid-Volume (sec.)	0.50	0.55	2.5

FIG. 2. Spirometric tracings obtained during expiration of maximal volume and rapidity. Note the placement of marks to indicate 25 per cent of the total deflection at each end.

widely observed with recording spirometers. Recordings of rapid expirations made by healthy men and by a patient who had emphysema are illustrated in figure 2. An 18-liter spirometer of the Benedict-Roth type was used at a drum speed of 8 mm. per second, with the carbon dioxide absorber and valves removed.

Although patients who have emphysema can develop a relatively large rate of flow for the initial volume expired, this rate cannot be maintained for the major part of the total expiration. In contrast, the normal person can maintain a fairly large rate of flow for the major part. However, both normal persons and patients had a relatively slow rate of flow toward the end of expiration. This suggested the possibility that increased ability to detect expiratory retardation might be obtained by a measurement which (*1*) excluded the early volume expired, which in any case might not be accurately recorded by conventional spirometers; and (*2*) excluded the last part of the volume expired, which was relatively retarded in both healthy persons and in patients. In other words, perhaps the expiratory slowing observed in patients of this type tended to be maximal during the midpart of expiration.

Partition of total expired volume: In order to test this possibility set forth immediately above, it was decided arbitrarily to separate the total expired volume into three parts, namely, the first quarter, the middle half, and the last quarter. Measurements were made of the mean rate of flow and of the duration of each of

TABLE 1

AVERAGE ABSOLUTE AND RELATIVE VALUES OF RATE OF FLOW AND DURATION OF SEVERAL PARTS OF MAXIMALLY RAPID AND DEEP EXPIRATION

Group	Age	Persons	Records		Parts of Total Expired Volume						Ratio of Mean Rates of Flow	
					First Quarter		Midhalf		Fourth Quarter			
			Spirometer	Pneumotachograph	Flow	Duration	Flow	Duration	Flow	Duration	Midhalf: First Quarter	Last Quarter: First Quarter
	years				*L./sec.*	*seconds*	*L./sec.*	*seconds*	*L./sec.*	*seconds*	*per cent*	*per cent*
Young men	28–39	15	10	9	5.6	0.23	4.4	0.59	0.7	2.3	83	14
Old men	47–79	12	21	—	4.2	0.24	2.9	0.64	0.4	2.7	76	9
Patients with emphysema	46–68	10	9	10	1.2	0.53	0.5	2.50	0.2	3.0	45	21

these parts on both spirometric and pneumotachographic recordings from healthy men and from men who had emphysema. All types of recording confirmed the same conclusion.

The summary of all of the data is given in table 1, which lists the average values for flow and duration and also the averages of ratios of the rates of flow during the middle and last phases to the rate during the first quarter. Both age groups of healthy men were able to maintain a rate of flow during the middle half which was more than 75 per cent of the rate during the first quarter. The patients who had emphysema, however, had a relatively greater retardation during the middle half than during either the first or last quarter, as shown both by low rates and prolonged duration of flow.

The accuracy of spirometric recording of the rate of flow and the duration of the middle-half volume was tested as follows.

A total of ten records was obtained from 6 healthy persons (3 men and 3 women) in whom the forced expiration was made through the pneumotachograph to the recording spirometer; the drum speed was 8 mm. per second for six records and 2.67 mm. per second for four records. Measurements were made of the mean rate of flow and the duration of the middle half and also of the percentage expired in 1 second, which has been widely recorded spirometrically by other workers.

The average midvolume measurements obtained by the spirometer (4.24 liters per second and 0.501 second) were not significantly different from those of the pneumotachograms (4.29 liters per second and 0.504 second). However, a small (3 per cent) but consistently greater amount expired in 1 second was recorded by the spirometer. This is a characteristic of this particular spirometer under these conditions. It should also be noted that it is not possible to exclude a small systematic error in the measurement of the flowmeter, which is calibrated with difficulty at such high rates of flow as occur during the first several tenths of a second.

Comparisons at two different drum speeds were made, with the intention of

sing a drum speed of 8 mm. per second to reduce the error of reading in studies which establish normal values and of using the standard commercial drum speed of 2.67 mm. per second for routine clinical testing, for which it is both convenient and sufficient. Although a faster drum speed has been used by various investigators, this does not increase the accuracy of measurement when this is limited mainly by the frequency-response characteristics of the spirometer itself.

After it had been determined that the spirometer could yield measurements of midvolume which had no systematic inaccuracy and were probably only slightly in error with regard to measurement of the percentage of the total expiration which was expired in 1 second, a more extensive study of healthy persons was begun.

Normal Values in Healthy Adults

Subjects: The subjects were 140 healthy adults who were considered, on the basis of their previous annual histories and results of physical examination (including roentgenograms of the thorax), to be free from cardiac or pulmonary disease. They were technical, clerical, or professional employees of the Mayo Clinic or students therein.

Procedure: Studies were done with the subject in the sitting position. The subject first was instructed, with demonstration, to make a maximal inspiration and then a maximal expiration. For this attempt, the subject was not instructed to expire with maximal rapidity. The subject was then instructed, with demonstration, to make an expiration of both maximal volume and rapidity; this was repeated in a minute or so unless additional attempts were necessitated by apparent lack of effort or understanding. A mouthpiece and nose clip were used, being connected by means of a Y tube through two parallel 120 cm. lengths of smooth bore tubing, 22 mm. in internal diameter, to the spirometer without valves or carbon dioxide absorber.

Measurements: Two marks were placed on the spirometric tracing, each at a point representing 25 per cent of the total deflection from the respective extreme (figure 2). A transparent plastic sheet with suitable scales (figure 3) was then placed over the tracing so that (*1*) its horizontal axis was exactly parallel to the horizontal base line of the tracing (the identical numbered scales on the sides facilitate this) and (*2*) an inscribed point *A* exactly overlay the upper (25 per cent) mark on the spirometric record. A transparent straightedge was then placed on the plastic sheet and aligned to pass through point *A* and the lower (75 per cent) mark on the spirometric record, and the corresponding slope was read on the lower horizontal scale. The lower scale was constructed most easily by making the vertical distance from point *A* to the horizontal scale equal to an arbitrary volume, such as 6 liters, and marking the units of the horizontal scale in appropriate values of seconds per liter. This gave a linear scale from which the values were readily converted to liters per second. The scale may be constructed to read liters BTPS (body temperature, ambient pressure, saturated) by using average local values for ambient temperature and barometric pressure; no important error is introduced if daily variations are not extreme. The duration in seconds of the period required to expire the midvolume, or the midexpiratory time, is obtained by dividing one half the total volume expired by the mean rate of flow of the midvolume.

The percentage of the total volume expired in 1 and in 3 seconds was determined by placing the inscribed point *C* over the point where the expiratory deflection began an abrupt descent. Lines *D* and *E* are at right angles to the base line at a horizontal distance from point *C* equal to the drum speed times one and times three, respectively, and indicate the volume expired during these intervals of time. It is not uncommon, just after the completion of maximal inspiration, for the subject to delay momentarily the start of the forced expiration. A small volume of gas may be expelled at this time over a variable period pre-

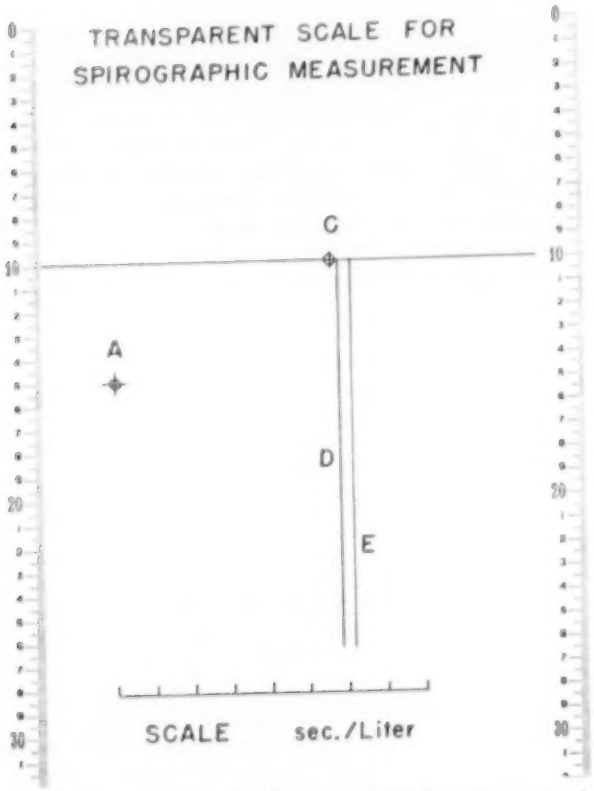


FIG. 3. Appearance of transparent plastic scale to facilitate measurement of spirograms.

ceding the start of the rapid expiration. If the 1-second period is started at the initial expiratory deflection, a small value will be obtained for the volume expired in 1 second. Records in which uncertainty existed about the initial expiratory point were excluded from the 1-second and 3-second measurements; such records were, however, satisfactory for measurement of midexpiratory flow. Difficulties in accurate definition of the starting point of the rapid expiration may arise not only from the subject's performance but also from the actual limitations of the spirometer in recording this brief period of high acceleration of volume (figure 1). The horizontal scales of figure 2 are constructed for a drum speed of 2.67 mm. per second, which was used for studies on patients. As already indicated, normal subjects were studied at a drum speed of 8 mm. per second and another scale was used which had a 2 second line in addition.[4]

[4] Due to the efforts of Dr. Ross Kory, Veterans Administration Hospital, Wood, Wisconsin, a plastic scale with an attached pivoted straightedge can be obtained from Presco Plastics, Inc., 121 North Broadway, Milwaukee, Wisconsin. This is suitable for measurements of the midvolume and also the usual spirometric measurements, including 1-second values and so forth, as they are recorded by the 13.5-liter respirometer, Warren E. Collins, 555 Huntington Avenue, Boston 15, Massachusetts. It also may be used with the 9-liter respirometer by alteration of one scale.

The results of this observation of normal adults are presented in table 2, and all volumes are body temperature, ambient pressure, saturated. The vital capacity, which was the largest volume of three or more maximal expirations, showed a decrease in persons who were more than fifty years of age. The mean value of maximal midexpiratory flow (MMF) was approximately 4.5 liters per second in men and 3.7 liters per second in women, a decrease occurring after fifty years of age in both sexes. The time required to expire the midvolume was approximately 0.6 second in men and 0.5 second in women, with no change with advancing age. The percentage of the vital capacity which was expired in 1 and in 3 seconds did not change with age in men, but a small reduction in the 1-second percentage was observed in older women.

Significant differences between sexes were observed in: volume of vital capacity; maximal midexpiratory flow; midexpiratory time; and percentage of the vital capacity expired in 1 and in 2 seconds. Correlations that were significant, but not strong, were found between MMF and the 1-second percentage in both men ($r = 0.50$) and women ($r = 0.61$), and between MMF and vital capacity as per cent of the predicted value (18) in men ($r = 0.44$) and women ($r = 0.31$). The weakness of these correlations is of some assistance in the evaluation of a patient who shows borderline values in different tests.

Reproducibility: In table 3 are given the results of comparisons between different observers measuring the same records; duplicate tests; and duplicate tests on the same persons on different days, one week to one month apart. The smaller numbers of persons for the 1-second and 3-second values for vital capacity indicate that at least one of the records was unsuitable for measurement because of uncertainty about the initial deflection. The only systematic differences noted were small increases of MMF on the second trial and the tendency of one observer to measure MMF approximately 3 to 4 per cent greater. However, considerable variation occurred from time to time with all of the measurements listed.

Clinical Application in Chronic Bronchopulmonary Disease

An example will be presented of the clinical utility of the measurement of maximal midexpiratory flow and midexpiratory time. The records in 100 adults were selected from the departmental files by alphabetic order of names. Fifty patients had primary diagnoses of bronchial asthma or chronic bronchitis, usually asthmatic, and 50 had primary diagnoses of pulmonary emphysema. All diagnoses were made on the basis of the histories; results of physical examination; the roentgenographic appearance of the thorax; and the results of tests of pulmonary function, which included measurement of maximal breathing capacity (MBC) and total lung capacity and its subdivisions, as well as other appropriate procedures. The group consisted of 15 women and 85 men, whose respective average ages were forty-nine and fifty-four years.

The number of these patients judged to present abnormal results of several ventilatory tests, all of which may be done with only a spirometer, is listed in table 4. The limits of normality correspond to two standard deviations from the mean, except as follows. The prediction formulas of Baldwin and associates (19)

TABLE 2

NORMAL VALUES: MAXIMALLY RAPID AND DEEP EXPIRATION

Sex	Mean Age, Years	Mean Height	Mean Surface Area	Persons*	Tests*	Vital Capacity†	Per Cent of Vital Capacity Expired			Maximal Midexpiratory Flow†	Midexpiratory Time†
							1 Second†	2 Seconds†	3 Seconds‡		
	range	*cm.*	*sq. meters*			*liters*				*L./sec.*	*seconds*
M	27.2 (20–29)	178.5	1.93	10 (7)	20 (14)	5.45	80.4 ± 4.1 (1.10)	95.0 ± 2.1 (0.57)	98.8 (96–100)	4.68 ± 1.10 (0.25)	0.59 ± 0.14 (0.03)
M	34.3 (30–39)	179.0	1.99	23 (13)	46 (25)	5.20	81.7 ± 5.6 (1.12)	93.4 ± 2.9 (0.58)	97.6 (92–100)	4.38 ± 1.00 (0.15)	0.61 ± 0.15 (0.02)
M	44.7 (40–49)	178.7	1.98	19 (16)	38 (31)	5.19	79.1 ± 9.8 (1.75)	93.8 ± 4.7 (0.84)	98.2 (91–100)	4.37 ± 1.10 (0.18)	0.60 ± 0.17 (0.03)
M	54.3 (50–59)	179.1	2.03	14 (14)	28 (24)	4.84	76.1 ± 6.7 (1.37)	91.2 ± 4.5 (0.92)	96.7 (91–100)	3.71 ± 1.00 (0.19)	0.67 ± 0.20 (0.04)
M	65.9 (60–79)	174.1	1.93	10 (9)	20 (17)	3.82	80.3 ± 7.1 (1.73)	93.9 ± 3.1 (0.76)	99.0 (94–100)	3.12 ± 0.80 (0.18)	0.60 ± 0.11 (0.03)
All men	(20–79)	—	—	(59)	(111)	—	79.4 ± 7.5 (0.71)	93.3 ± 3.3 (0.37)	97.9 (91–100)	—	0.61 ± 0.16 (0.013)
F	21.9 (17–29)	164.6	1.61	33 (27)	66 (47)	3.48	87.5 ± 7.7 (1.13)	98.4 ± 2.4 (0.35)	99.9 (98–100)	3.60 ± 0.88 (0.11)	0.48 ± 0.11 (0.01)
F	37.2 (30–45)	164.3	1.64	20 (17)	40 (34)	3.30	88.2 ± 7.1 (1.22)	98.1 ± 2.8 (0.48)	99.9 (98–100)	3.77 ± 0.88 (0.14)	0.44 ± 0.11 (0.02)
F	52.5 (46–62)	160.1	1.63	11 (10)	22 (19)	3.06	83.0 ± 5.8 (1.75)	96.3 ± 2.8 (0.65)	99.2 (95–100)	2.97 ± 0.59 (0.13)	0.50 ± 0.09 (0.02)
All women	(17–62)	—	—	(54)	(100)	—	86.9 ± 7.4 (0.74)	97.9 ± 2.7 (0.27)	99.8 (95–100)	—	0.47 ± 0.11 (0.01)

* Numbers in parentheses indicate persons examined and tests done for 1-second, 2-second and 3-second percentages of vital capacity.

† Values listed are mean ± S.D. (S.E. mean).

‡ Values listed for 3-second percentage are mean and range (latter in parentheses).

TABLE 3

REPRODUCIBILITY OF SPIROGRAPHIC TESTS ON HEALTHY SUBJECTS

Comparisons	Pairs	Mean Difference	S.D., ±	S.E. Mean, ±
Same records: MMF measured by two observers	35	0.108 liter/second*	0.26	0.044
Same day: first test minus second test on a subject				
MMF	140	−0.143 liter/second*	0.53	0.045
Midexpiratory time	140	0.0125 second	0.08	0.007
1-second per cent of vital capacity	104	−0.84 per cent	6.70	0.658
3-second per cent of vital capacity	104	0.125 per cent	1.40	0.137
Twenty subjects				
Day 1: first test minus second test				
MMF	20	−0.12 liter/second	0.56	0.12
Midexpiratory time	20	0.022 second	0.08	0.02
Day 2: first test minus second test				
MMF	20	−0.21 liter/second	0.76	0.17
Midexpiratory time	20	0.026 second	0.17	0.04
Average of day 1 minus average of day 2				
MMF	20	−0.04 liter/second	0.73	0.16
Midexpiratory time	20	0.02 second	0.10	0.02
1-second per cent of vital capacity	13	−1.6 per cent	4.90	1.40
3-second per cent of vital capacity	13	−1.0 per cent	2.70	0.80

* Significant difference.

TABLE 4

COMPARISON OF SPIROGRAPHIC TESTS ON 100 PATIENTS WITH BRONCHITIS OR EMPHYSEMA

Test	Normal Limit	Patients Normal by Test	Number of Same Patients Normal by MMF*
Vital capacity	75 per cent predicted		
	Baldwin formula†	57	12
	Bateman formula‡	31	9
3-second per cent of vital capacity	Women 98 per cent§ Men 93 per cent‖	35	12
MBC	65 per cent predicted	24	7
	75 per cent predicted	20	6
	Baldwin formula†		
1-second per cent of vital capacity	Women 72 per cent Men 65 per cent	22	11
Midexpiratory time	150 per cent predicted	13	11
MMF	50 per cent predicted	13	—

* The numbers in the right-hand column are less than 13 because several patients having MMF values of 50 to 60 per cent of predicted had abnormal values for another test.

† Baldwin and associates (19).

‡ Bateman (18).

§ Ninety-five per cent of observations were between 98 and 100 per cent, inclusive.

‖ Ninety-five per cent of observations were between 93 and 100 per cent, inclusive.

for vital capacity and MBC were derived from several age groups within which the coefficients of variation were 15 to 20 per cent, suggesting a lower limit of normal of approximately 65 per cent of the predicted value. The results of using a lower limit of 75 per cent are also given. Since the present subjects were examined in the sitting position rather than supine, as was used in determining the formula for vital capacity, the limit was increased to 75 per cent of the predicted value. The normal values for MMF, using appropriate age, and for midexpiratory time and the 1-second and 3-second values were obtained from table 2.

It is evident that the least number of persons had normal values for MMF and for midexpiratory time, followed by those who had normal values for the 1-second percentage of vital capacity and for MBC. The results of such comparisons, however, depend on the type and severity of disease present. Results of all tests were abnormal in patients who had severe emphysema. In patients who had less pronounced impairment, the vital capacity, MBC, and 3-second percentage of vital capacity often failed to detect abnormalities which were readily apparent by the other tests. As already noted, the patients were selected arbitrarily from two primary diagnostic categories to illustrate the relative sensitivity of the various tests without other sampling bias.

In table 5, the data are grouped according to the values for MMF. The best result of two or more trials of each test was used. In this table the well-known fact is illustrated that considerable degrees of obstructive pulmonary disease may not be accompanied by a reduced vital capacity because the patient can expel a large volume if enough time is taken. A period of 3 seconds is sufficiently long so that patients who have moderate impairment are able to expire a normal percentage. The Baldwin formula for prediction of vital capacity yields normal values which are too small, as noted by the present writers and investigators. Consequently, with the use of the formula, there is a failure to detect moderate reductions. The Bateman formula, which is based on the cube of height, is more useful in the present writers' experience.

TABLE 5

MEAN VALUES OF VENTILATORY TESTS ON 100 PATIENTS, GROUPED BY MAXIMAL MIDEXPIRATORY FLOW

Per Cent of Normal MMF	Patients	Per Cent of Normal					Vital Capacity‡	
		MMF	Midexpiratory Time	Vital Capacity		MBC*	1 Second Per Cent	3 Second Per Cent
				Baldwin*	Bateman†			
>50	13	61	127	100	83	76	74	98
41–50	8	45	192	101	85	61	66	98
31–40	11	36	222	101	82	73	62	95
21–30	15	24	278	89	72	54	57	88
11–20	33	14	390	71	58	37	47	80
0–10	20	8	500	63	51	25	43	76

* Baldwin and associates (19).

† Bateman (18).

‡ Volume expired on maximally deep and rapid effort.

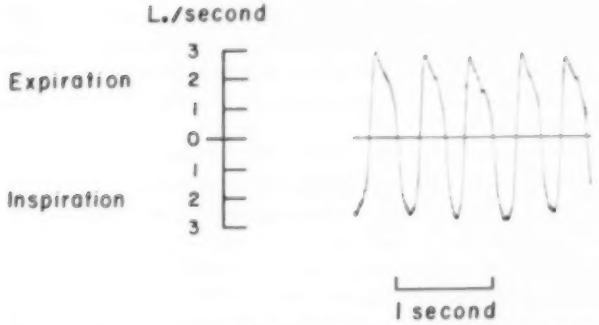


FIG. 4. Pneumotachogram obtained during determination of maximal breathing capacity in the same patient with emphysema whose tracing is shown in figure 1, lower panel.

The failure of the MBC to detect moderate impairment was caused to a considerable degree by its failure to decrease in proportion to expiratory retardation, as shown in table 5. Both the spirographic records (figure 2) and the pneumotachograms of patients who had emphysema (figure 1) show that a certain amount of gas can be expelled at relatively high rates of flow. During measurement of MBC, patients use this amount of gas as the major part of their tidal volume, thereby achieving a near maximal average rate of flow. This is illustrated in figure 4, which is a pneumotachogram obtained during measurement of the MBC of the same patient whose forced maximal expiration is shown in figure 1.

Similar records have been obtained from 9 other patients who had emphysema. The midvolume, which can be expired much less rapidly, is not used by these patients in performing a MBC effort. Measurement of the 1-second percentage of vital capacity fails to detect abnormalities when moderate reductions of both volume and flow coexist in a relationship which leaves the 1-second percentage relatively unchanged.

It might be asked whether the MMF yielded values which were falsely high or low in relation to the patient's impairment. The answer depends, of course, on the standards used, but is of particular importance when the established tests, such as vital capacity and MBC, fail to show an abnormality. A review of the clinical findings and the results of all tests of pulmonary function, which also included determination of residual volume and total lung capacity and of the alveolar concentration of nitrogen after the patient breathed oxygen for 7 minutes, indicated that all patients who had abnormal values for MMF also had other abnormalities.

Of the 13 patients who had values for MMF greater than the lower limit of 50 per cent of normality, 12 had values which were 65 per cent of normal or less. Other evidence also indicated that impairment was present in these patients, although it was of only moderate degree. The remaining patient had a MMF which was 91 per cent of normal, showed normal values for all other tests of pulmonary function, and had mild chronic bronchitis on clinical examination. In chronic asthmatic bronchitis or emphysema of moderate degree, the MMF

appears to bear a closer relationship to dyspnea than does the MBC. Although 24 of the 100 patients had values for MBC (directly measured) greater than 65 per cent of the predicted value, 20 of the 24 complained of dyspnea. Only one of these 20, a patient who had a large functional element, had a MMF greater than 64 per cent of normal.

The relatively large retardation of the midexpiratory volume noted previously (table 1) also was demonstrated in this group of patients. In table 6 it may be noted that the retardation also increased with the severity of impairment, as judged by vital capacity or MBC.

The reproducibility on duplicate trials of several tests on the 100 patients is shown in table 7. A small mean increase of MBC was observed on the second trial. With all procedures, the differences between tests are sufficiently large that at least duplicate trials are advisable for diagnostic categorization.

Three conventional measurements of vital capacity without an attempt at rapidity were made prior to the duplicate trials with rapidity. In 26 of the 100

TABLE 6

RELATIVE VALUES OF RATE OF FLOW DURING SEVERAL PARTS OF EXPIRATION IN 100 PATIENTS, GROUPED BY MAXIMAL BREATHING CAPACITY AND BY VITAL CAPACITY*

Grouping	Patients	Per Cent of Normal	Ratios of Mean Rates of Flow	
			Midhalf: First Quarter	Last Quarter: First Quarter
MBC	50	29 ± 1.0	0.40 ± 0.013	0.17 ± 0.007
	50	66 ± 3.0	0.62 ± 0.030	0.15 ± 0.010
Vital capacity	50	53 ± 1.3	0.43 ± 0.020	0.17 ± 0.010
	50	81 ± 1.8	0.60 ± 0.020	0.14 ± 0.010

* Values listed are the mean and its standard error.

TABLE 7

REPRODUCIBILITY OF SPIROGRAPHIC TESTS ON 100 PATIENTS

	First Test* Minus Second Test			
	MBC	1 Second Per Cent of Vital Capacity	MMF	Midexpiratory Time
	L./min.	*per cent*	*L./sec.*	*seconds*
Mean difference	−2.10†	−1.20	0.001	−0.040
S.D., ±	7.40	7.00	0.170	0.560
S.E. mean, ±	0.74	0.70	0.017	0.056
	First Test:Second Test			
Mean ratio	0.950†	0.980	1.003	1.002
S.D., ±	0.160	0.140	0.240	0.240
S.E. mean, ±	0.016	0.014	0.024	0.024

* Average values for first test were: MBC = 45.3 liters per minute; 1-second per cent of vital capacity = 51.0; MMF = 0.89 liter per second; midexpiratory time = 2.07 seconds.

† Significant difference.

patients, chiefly in the more severely impaired group, the largest volume expired rapidly was less than 90 per cent of the largest volume expired slowly. This indicates that a distinction must be made in patients of this type between the conventional vital capacity and the total volume expired during a maximally rapid effort.

DISCUSSION

The normal percentages of the vital capacity expired in 1, 2, and 3 seconds are similar to the average values reported by Gaensler (8). The Gaensler data were aggregated for both sexes, most of whom were young adults, and did not include analysis of the variations among subjects. The significant difference found between sexes in the present study (table 2) was not observed by Drutel and Dechoux (20) in subjects in the third decade of life. The present data fail to show a decrease of the percentage expired in 1 second by older men, a finding which has been noted by several French writers (20–22). Although the older subjects of the present study were, generally, vigorous for their age and probably highly motivated, they exhibited a decline in vital capacity with age to an extent similar to that noted by the French writers, as indicated by values for vital capacity divided by the bodily surface area in square meters.

A variety of descriptions have been made of the shape of the pattern of expiratory flow as recorded by spirometers. It often has been said to be a straight line followed by a curve, the two parts being demarcated by a "critical point" which is sometimes difficult to establish (5, 7, 23, 24). A straight line on a spirometric record implies that the rate of flow is constant. In the present study, this type of spirometric record has been obtained from normal subjects and from patients who had emphysema. However, pneumotachograms of several of these persons have been like those illustrated in figure 1 and have not shown an initial period of sustained constant rate of flow. However, such periods have been observed occasionally in pneumotachograms of normal persons when the effort was somewhat submaximal. The early straight line in general is probably a damped recording of the initial "triangular" part of the pattern of flow. Straight lines were not observed by Bernstein (15) in studies in which an improved spirometer was used. In patients who have emphysema, the "critical point" is related to the "elbow" of the descending limb of the pattern of flow, that is, the rate of decrease of flow more or less abruptly lessens. This can be seen in the reports of Roche and Thivollet (6) and Dayman (25), and it has been seen to a variable degree in the pneumotachograms taken during both forced total expiration and MBC expiration on patients with emphysema in the present study.

Spirometric records also have been described as having the form of a hyperbola (26, 27) or of a single (28) or double (28, 29) exponential. Although certain spirometric recordings can be more or less well approximated by any of these descriptions, this is partially the result of variable degrees of spirometric distortion. The presence of large accelerations of volume, both positive and negative, in expiratory pneumotachograms of normal persons and patients who have pulmonary disease has been repeatedly observed for many years (30–33) and is inconsistent with strict application of any of these mathematical expressions.

Several reasons exist for directing attention to the shape of the pattern of expiratory flow, as accurately recorded by pneumotachograms. Many measurements have been proposed on the basis of spirometric recordings. These include the volume expired in 3.2 (8) and 1.5 (24) seconds, as well as in 1.0 (3, 6, 8), 0.75 (24), 0.6 (27), 0.5 (6, 34), and 0.43 (27) second. Other measurements have been the total mean rate of flow (2, 35), the maximal rate of flow (36, 37), and the time required to expel 63 per cent of the total volume (27). Many reasons have been given for selection of these different methods, but emphasis has been placed frequently on the relation between the pattern of expiratory flow and the MBC. In fact, Tiffeneau's procedure, which consists of multiplying the volume expired in 1 second by a factor of 30 to obtain the "indirect maximal breathing capacity," is widely used in France.

Various studies have shown that the mean rate of flow of the early part (1 second or less) of the forced expiration is correlated to varying degrees with the MBC as determined directly; Kennedy (24) found an approximate 1:1 relationship, using the volume expired in 0.75 second. This is not unexpected, since this period includes the maximal rate of expiratory flow, and the MBC in patients who have chronic pulmonary disease is usually limited by the expiratory rather than inspiratory flow. However, the pattern of flow during the early part of expiration is most liable to errors of measurement by spirometry because of the large accelerations of volume. Even if a measurement could be obtained from the single expiration which would be equal to the MBC measured directly, it would have disadvantages as well as advantages. It would, with a simpler procedure, obtain an "indirect" MBC. This is attractive because there is much clinical experience which shows the MBC to be useful, and no doubt it explains the numerous studies of the correlation of the MBC with various segments of a single forced expiration. Bernstein and Kazantzis (38) recently have questioned the utility of such efforts on statistical and other grounds.

It is not the present purpose to enter the controversies about the execution, terminology, or interpretation of the MBC, but it is regarded as a clinically useful test which is not synonymous with the single rapid expiration. A principal limitation of the MBC is that the patient's obstructive impairment tends to be underestimated. Measurement of chiefly the early, relatively high rates of flow, by either the observer's selection on the record of a single expiration or the patient's selection during a MBC effort, ignores the inability to maintain a high rate of flow. The relatively large retardation found during midexpiration is consistent with current views on the mechanism of expiratory obstruction in emphysema (25, 39, 40), which views also indicate the reasons why a maximally rapid expiratory effort is such a useful test. Resistance to expiratory flow, to some degree in normal persons and to a greater extent in patients who have pulmonary emphysema, increases, first, as the volume of the lungs decreases, with an accompanying reduction in elastic pulmonary tension and in the diameter of the airway; and, second, as voluntary expiratory effort is increased. In the latter situation, there is an accompanying rapid development of a high gradient of pressure between alveoli and airway, which in turn exerts a collapsing

force on the airway. The testing procedure of maximally rapid and deep expiration emphasizes the combined effect of these two obstructive factors, and the resulting effect apparently achieves a maximal deviation from normality during expulsion of approximately the midvolume.

SUMMARY

A ventilatory test of widely acknowledged utility is the performance of a maximally rapid and deep expiration. Disagreement exists on the characteristics of the pattern of flow and on the method of measurement.

Study of pneumotachograms of healthy adults and of patients who had pulmonary emphysema confirmed the presence of large accelerations of volume during the first several tenths of a second; these accelerations make accurate recording by spirometers uncertain. However, the mean rate of flow and the time required for expiration of the middle 50 per cent of the total volume expired (maximal midexpiratory flow and midexpiratory time) could be recorded accurately by a spirometer of the conventional Benedict-Roth type. The expiratory retardation found in adult patients with chronic bronchitis or emphysema was found to be most pronounced during expulsion of the midpart of a maximally deep and rapid expiration.

Normal standards for maximal midexpiratory flow and midexpiratory time, and measurements of the percentage of the total volume expired in 1 and 3 seconds, were obtained from a study of 140 healthy persons of both sexes and of various ages. An example of the clinical utility of these measurements, which are simply and rapidly made, is presented, based on observations on 100 patients who had chronic bronchopulmonary disease. Maximal midexpiratory flow and midexpiratory time were found to be more sensitive as detectors of obstructive impairment than were previously used tests, including vital capacity, the percentage of vital capacity expired in 1 and in 3 seconds, and maximal breathing capacity. The last-named group of tests tends to underestimate the extent of obstructive impairment.

In more than two years of daily clinical use, the measurements of maximal midexpiratory flow and midexpiratory time have proved to be simple and valuable. They can be determined with sufficient accuracy by means of spirometers and drum speeds which are commercially available. They are useful additions to the customary ventilatory measurements of vital capacity, 1-second percentage of vital capacity, and maximal breathing capacity, particularly when the last-named tests give borderline results.

SUMARIO

Corriente Mesoespiratoria Máxima

Una prueba ventiladora de utilidad ampliamente reconocida es la ejecución de una espiración rápida y profunda a su máximo, mas existe desacuerdo acerca de las caracteristicas de la corriente y del método de medición.

El estudio de neumotacogramas de adultos sanos y de enfermos que han padecido enfisema pulmonar confirmó la presencia de grandes aceleraciones del volumen durante las primeras varias décimas de segundo; esas aceleraciones prestan incertidumbre al registro

exacto por medio de espirómetros. Sin embargo, la velocidad media de la corriente de aire y el tiempo requerido para la espiración del 50 por ciento que representa el segmento medio del volumen total espirado (corriente mesoespiratoria máxima y tiempo mesoespiratorio) pudieron ser registrados exactamente con un espirómetro del tipo corriente de Benedict-Roth. El rotardo espiratorio observado en los adultos que padecen de bronquitis o enfisema crónicos resultó ser más acentuado durante la expulsión de la porción media de una espiración llevada al máximo de profundidad y rapidez.

Por medio del estudio de 140 personas sanas de ambos sexos y de varias edades, se obtuvieron pautas normales para la corriente mesoespiratoria máxima y mediciones del porcentaje del volumen total espirado en uno y en tres segundos. Ofrécese un ejemplo de la utilidad clínica de estas mediciones, que se ejecutan sencilla y rápidamente, a base de observaciones en 100 enfermos que tenían afección broncopulmonar crónica. La corriente mesoespiratoria máxima y el tiempo mesoespiratorio resultaron ser descubridores más sensibles de la insuficiencia oclusiva que las pruebas usadas anteriormente, comprendiendo la capacidad vital, el porcentaje de la capacidad vital espirado en uno y en tres segundos y la capacidad respiratoria máxima. Este último grupo de pruebas tiende a rebajar la extensión de la insuficiencia oclusiva. En más de dos años de empleo clínico diario, las mediciones de la corriente mesoespiratoria máxima y del tiempo mesoespiratorio se han mostrado sencillas y valiosas. Cabe determinarlas con suficiente exactitud por medio de espirómetros y de velocidades de tambor obtenibles en el comercio. Constituyen, pues, adiciones útiles a las habituales mediciones ventiladoras de la capacidad vital, del porcentaje de capacidad vital en un segundo y de la capacidad respiratoria máxima, en particular cuando estas últimas pruebas dan resultados indecisos.

Resume

Débit mi-expiratoire maximum

Un test de ventilation d'utilité notoire consiste dans la pratique de l'expiration de rapidité et de profondeur maxima. Il existe un désaccord quant aux caractéristiques de la courbe du débit et à la méthode de mensuration.

L'étude des pneumotachogrammes d'adultes sains et de malades affectés d'emphysème pulmonaire confirmait la présence de grandes accélérations de volume pendant les premiers dixièmes de seconde; ces accélérations prévenaient la précision de l'enregistrement spirométrique. Toutefois, le taux moyen du débit et le temps requis pour l'expiration de 50% du volume total expiré (débit maximum mi-expiratoire et temps mi-expiratoire) pouvait être enregistré avec précision à l'aide du spiromètre de type Benedict Roth classique. Le ralentissement expiratoire ayant été observé chez le malade adulte atteint d'emphysème chronique ou de bronchite chronique a été trouvé le plus accentué au cours de l'expulsion de la portion moyenne d'une expiration de rapidité et de profondeur maxima.

Les valeurs normales pour le débit mi expiratoire maximum et le temps mi-expiratoire, ainsi que les déterminations du pourcentage du volume total expiré en une et en trois secondes, résultent des recherches effectuées chez 140 sujets sains des deux sexes et d'âges variés. Un exemple de l'utilité clinique de ces déterminations, dont la pratique est simple et rapide, est fondé sur les observations faites chez 100 patients atteints d'affections broncho-pulmonaires chroniques. Le débit mi-expiratoire maximum et le temps mi-expiratoire maximum ont été trouvés plus sensibles comme indicateurs d'altérations obstructives que les tests antérieurement en usage, y compris la capacité vitale, le pourcentage de capacité vitale exhalé en une et trois secondes et la capacité respiratoire maxima. Le dernier groupe des tests mentionnés est susceptible d'entraîner une sous-estimation de l'étendue des lésions obstructives. Au cours de plus de deux années d'usage quotidien en clinique, les mensurations du débit mi expiratoire maximum et du temps mi-expiratoire maximum ont démontré leur valeur et leur simplicité. Elles peuvent être déterminées avec une précision suffisante à l'aide des spiromètres et des tambours enregistreurs du commerce. Ces mesures complètent utilement les déterminations classiques de ventilation de la capacité vitale, du

pourcentage par seconde de la capacité vitale et de la capacité respiratoire maxima, en particulier quand ces derniers tests donnent des résultats limites.

Acknowledgments

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SPONTANEOUS PNEUMOTHORAX[1, 2]

A Review with the Results of Pulmonary Resection in Nineteen Patients

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INTRODUCTION

The present report is concerned with an analysis of the treatment of 52 patients with spontaneous pneumothorax who were seen at the State University of Iowa Hospitals between the years 1938 and 1955. During the last six years, 19 of these patients have been subjected to various types of pulmonary resection for the treatment of the spontaneous pneumothorax. Until recently, physicians have considered spontaneous pneumothorax a not too dangerous disorder and have approached the management of the problem very conservatively.

MANIFESTATIONS OF SPONTANEOUS PNEUMOTHORAX

The sudden collapse of the lung due to a spontaneous pneumothorax is usually accompanied by severe chest pain and shortness of breath. The sudden pain may occasionally be confused with that due to myocardial ischemia, dissecting aortic aneurysm, spontaneous rupture of the esophagus, and other painful intrathoracic emergencies. The history of previous similar episodes, however, should make one suspect the possibility of a spontaneous pneumothorax. In the present series, 90 per cent of the patients had a dramatically sudden and severe onset of the symptoms. A more insidious onset has been observed and was present in 10 per cent of the patients. Some of them presented themselves for medical care with pneumothoraces of weeks' or months' duration (figure 1).

Spontaneous pneumothorax occurs in patients of all ages, but the highest incidence is in the early and middle age groups (figure 2). This not uncommon disorder occurs usually in otherwise healthy persons. Until two decades ago it was erroneously linked with pulmonary tuberculosis (1, 2, 3), but during recent years spontaneous pneumothorax unrelated to tuberculosis has been recognized as a separate clinical entity (4, 5). Hayashi (6), in a report written in 1915, described the mechanism of production of a spontaneous pneumothorax. He demonstrated on autopsy material a valvelike action of the scarred peribronchiolar tissue at the base of subpleural vesicles finally resulting in rupture of the subpleural blebs into the pleural space. This concept was corroborated by Fischer (7) in 1922 who also suggested that surgical intervention might be indicated for this disorder. Neither of the two writers was able to explain the reason for the formation of the scar tissue and of the subpleural blebs. Kjergaard in 1932 reported fifty-one patients

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[2] Presented before the Medical Session, as part of *Section 7A*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 23, 1955.

with spontaneous pneumothorax. He also pointed out that this disease has no etiologic connection with active pulmonary tuberculosis (4, 8).

Although most reports show a preponderance of spontaneous pneumothoraces in the male, in the present series 43 patients (82 per cent) were males and 9 (18 per cent) were females. As far as could be elicited, 23 of the 52 patients gave a history at the time of admission of a total of 106 previous pneumothoraces. The previously experienced episodes occurred 75 times on the same side which was involved at the time of admission to the hospital. The contralateral side was involved 23 times, and on 8 occasions the patients experienced previous bilateral

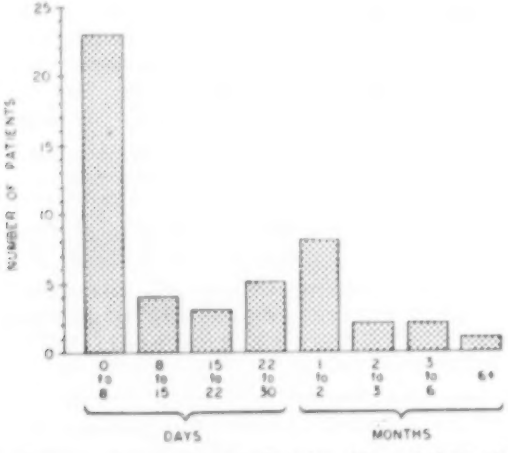

FIG. 1. Duration of pneumothorax prior to admission. Most of the patients presented themselves on the first day of onset of symptoms.

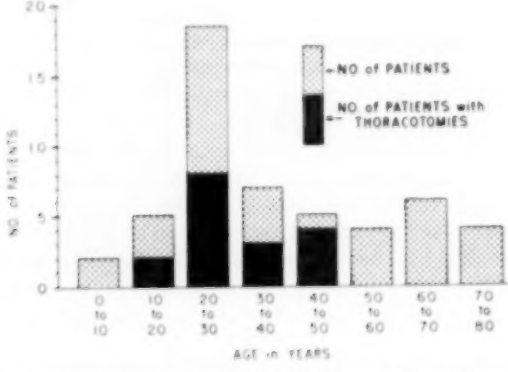

FIG. 2. Demonstrating the greatest incidence of spontaneous pneumothorax in the twenty- to fifty year age span and also the age incidence in which thoracotomy and resection of the subpleural blebs were employed.

pneumothoraces (9, 10, 11). The distribution of the pneumothoraces as to side involved at the time of admission was, as one might expect, rather equal. Bilateral involvement was present at the time of admission in 5 cases (12) (figure 3). The duration of the pneumothorax prior to admission varied from a few hours to more than six months. The greatest number of patients were seen during the first few hours after onset (figure 1). Of the 52 patients, 42 (84 per cent) presented themselves with either a complete or severe tension pneumothorax. Only 10 patients were seen with marginal collapse of moderate degree. Severe accompanying hemothorax was observed in 3 patients (6 per cent) (13). The bleeding in those patients was of sufficient degree to cause severe shock (figure 4).

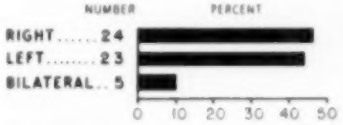

FIG. 3. Showing distribution of the side involved at the time of admission to the hospital.

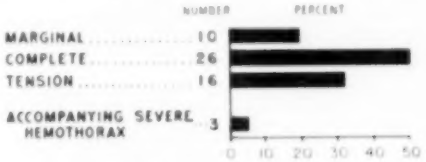

FIG. 4. Demonstrating the degree of pneumothorax encountered.

NON-RESECTIONAL TREATMENT

Initial and marginal pneumothoraces have been treated by needle aspiration in 7 of the patients of the present series. In the early years of the study, many patients were managed without the employment of any active therapy even though they presented themselves with complete pneumothoraces. The re-expansion of the lung in this group as far as can be ascertained has been very delayed. Many of these patients were discharged with incompletely expanded lungs. For more severe degrees of initial pneumothorax, intercostal underwater seal drainage without suction was employed (14, 15). This was the case in 12 of the 52 patients in this series (figure 5). The writers have not used other methods of therapy such as phrenic crush, closed pneumonolysis, poudrage, instillation of blood or sclerosing material into the pleural space (9, 14, 16, 17).

THORACOTOMY AND RESECTION

It is believed that in patients with repetitive or severe pneumothoraces a primary thoracotomy is indicated (17–21). Complications such as hemopneumothorax or severe tension pneumothorax with the presence of a large bronchopleural fistula, often held open by pleural adhesions, are more easily and safely managed by open surgical intervention.

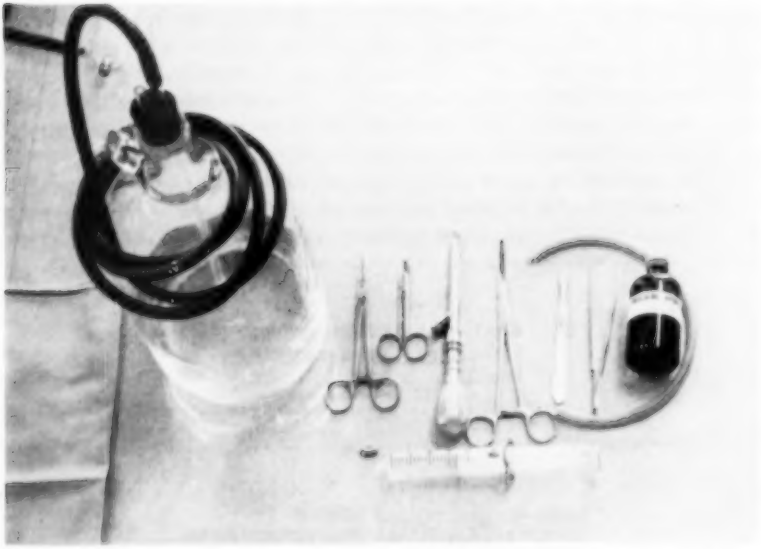

FIG. 5. Illustrating the simple equipment necessary to carry out underwater seal drainage.

In the present series, thoracotomy and resection of the subpleural blebs were carried out in 19 patients. Each of these patients exhibited ruptured subpleural blebs and bronchopleural fistulae. Most of them also had adhesive bands between the involved lung and adjacent chest wall. All of the blebs were located in the apical segments of the upper lobes except in two instances. It was possible to perform a simple wedge excision of the involved tissue in 16 patients while segmental resection was necessary twice and lobectomy was employed in one patient (table 1).

TABLE 1

SPONTANEOUS PNEUMOTHORACES

Demonstrating Type of Management and Average Hospital Stay of the 52 Reported Patients

Type of Management	Number of Patients	Average Length of Hospital Stay
		days
Thoracotomy and resection	19	12
Wedge excision	16	
Segmental excision	2	
Lobectomy	1	
Underwater seal drainage	12	14
Needle aspiration of air	7	14
Observation only	14	21

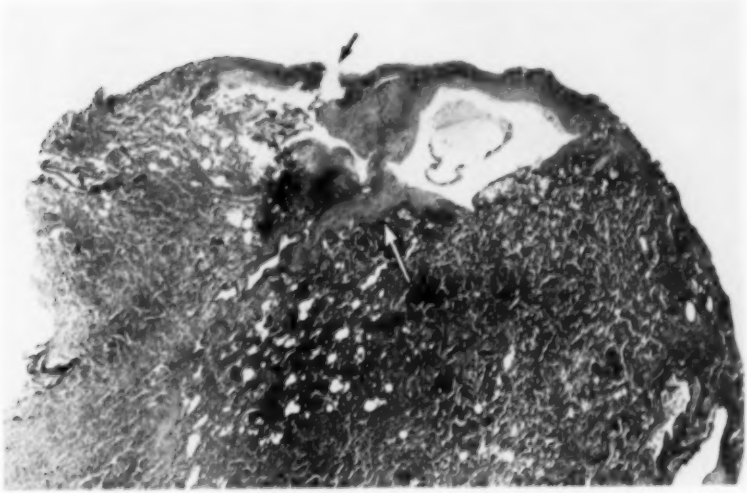

FIG. 6. Photomicrograph of excised ruptured subpleural bleb demonstrating the non-specific peribronchiolar scarring and the bronchiolar pleural fistula (see arrows).

FIG. 7. Roentgenogram showing distended subpleural bleb present in the apical portion of the right upper lobe (see arrow). The remaining lung is atelectatic.

FIG. 8. Roentgenogram showing hemopneumothorax. Note the large distended subpleural bleb visible above the fluid level (*see* arrow).

Mechanism of spontaneous pneumothorax: The basic pathophysiology producing spontaneous pneumothorax has been described in the past by a number of writers (4, 6–8, 19, 20, 24). All have noticed the localized subpleural nonspecific scarring of the peribronchiolar tissue, the formation of interstitial emphysema resulting in elevation of the overlying pleura, and subpleural cyst

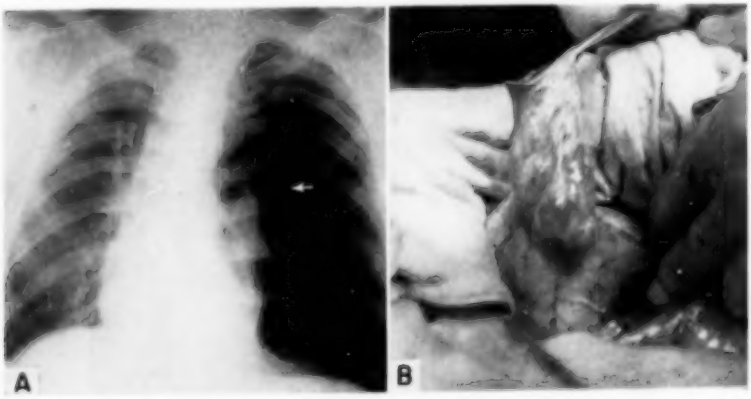

FIG. 9. *A.* Chest roentgenogram demonstrating the completely atelectatic left lung and the distended subpleural blebs (*see* arrow). *B.* Operative photograph of the large subpleural bleb located in the superior segment of the left lower lobe.

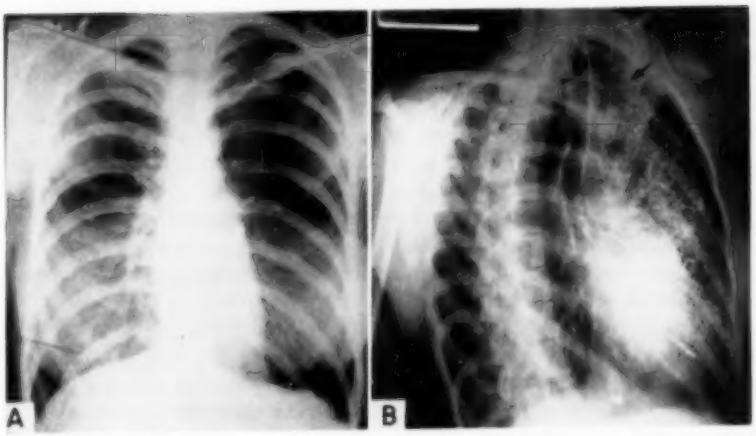

FIG. 10 *A*. Roentgenogram of the chest with a left marginal pneumothorax. Subpleural blebs are not visible. *B*. Bronchogram demonstrating the apical subpleural blebs. Note the absence of lipiodol in the lung parenchyma in the area of bleb formation (see arrow).

formation (figure 6). The connection of the subpleural bleb with an air-containing bronchiole permits the progressive distention of this bleb on the basis of an obstructive one-way valve mechanism. Filling of the blebs takes place during the expiratory phase of respiration while positive pressures are present within the bronchial tree. This finally leads to disruption of the bleb with formation of a bronchiolar-pleural fistula.

From the mechanics of spontaneous pneumothorax, it is readily understandable why nonexcisional therapy, which permits the lung to re-expand but does not remove the underlying causative factors, will lead to a high incidence of recurrence. In the presence of collapse and atelectasis of the normal lung tissue in a complete pneumothorax, one can frequently visualize the fully distended subpleural bleb in the chest roentgenograms or during chest fluoroscopy (20). This was the case in 14 of the 19 patients in the present series who were subjected to thoracotomy (figures 7, 8, 9*A* and *B*). Bronchograms (22) occasionally will assist in outlining the subpleural blebs, due to lack of filling of the peripheral bronchial tree in the involved areas with the contrast material (figures 10*A* and *B*).

Course following resection: Thoracotomy and excision of the ruptured subpleural blebs resulted in a short hospital stay with no morbidity or mortality. All patients were discharged with completely expanded lungs. Recurrences of pneumothoraces have not been observed in patients treated in this manner.[5] In contrast, the patients treated with intercostal underwater seal drainage, needle aspiration, or those only observed experienced longer hospital stays and a high recurrence rate (4, 15, 23, 24). In addition, three deaths occurred in this non-resected group; two were due to untreated tension pneumothoraces and

[5] The longest period of observation extended over the last six years.

one was due to inadequate intercostal drainage in a patient with bilateral pneumothorax.

SUMMARY

An analysis of 52 patients with spontaneous pneumothoraces has been presented. Nineteen of these patients have been subjected to thoracotomy and resection of ruptured subpleural blebs. Active pulmonary tuberculosis has not been found to be an etiologic factor.

It is believed that repetitive and severe tension pneumothoraces, as well as hemopneumothoraces, should be treated by immediate exploratory thoracotomy. Visualization of subpleural blebs in a spontaneous pneumothorax in chest roentgenograms also constitutes an indication for their excision.

Underwater seal drainage may be employed in initial and minimal degrees of pneumothorax. It also must be used as an emergency measure in patients with tension or bilateral pneumothoraces. Needle aspiration and expectant management seldom result in prompt and complete re-expansion of the lung. The incidence of recurrence is high with this latter type of treatment. The non-resectional therapy resulted in a mortality of 5.7 per cent in this series of patients.

Thoracotomy and excision of ruptured subpleural blebs provide for eradication of the lesion and result in prompt and complete expansion of the lung. This form of treatment has prevented further recurrences of pneumothorax.

SUMARIO

La Resección Pulmonar en el Neumotórax Espontáneo

Preséntase un análisis de 52 enfermos con neumotórax espontáneo. Diecinueve de ellos han sido tratados con la toracotomía y la resección de flictenas subpleurales desgarradas. La tuberculosis pulmonar activa no resultó ser un factor etiológico. Al parecer de los AA., los neumotóraces inflados repetidos y graves, e igualmente los hemoneumotóraces, deben ser tratados con la toracotomía exploradora inmediata. La visualización en las radiografías torácicas de flictenas subpleurales en un neumotórax espontáneo también constituye una indicación para la excisión de las mismas. En el neumotórax inicial y el mínimo, puede emplearse el drenaje cerrado bajo agua, que debe también usarse como medida de urgencia en los enfermos que muestran neumotóraces inflados o bilaterales. La aspiración con aguja y la asistencia expectativa rara vez logran la reexpansión rápida y total del pulmón, siendo alta la incidencia de recurrencias con esa forma de tratamiento. La terapéutica expectativa dió por resultado una mortalidad de 5.7 por ciento en esta serie de enfermos. La toracotomía y la excisión de las flictenas subpleurales rotas obtienen la erradicación de la lesión y dan por resultado la expansión pronta y completa del pulmón. Esta forma de tratamiento ha impedido nuevas recurrencias.

RESUME

Résection pulmonaire dans le pneumothorax spontané

Présentation de l'analyse de 52 cas de pneumothorax spontané. Dix-neuf de ces malades ont été soumis à la thoracotomie et à la résection des bulles sub-pleurales rompues. La tuberculose pulmonaire en activité ne représentait pas le facteur étiologique. L'auteur est d'avis que les pneumothorax à répétition et sous tension marquée, ainsi que les hémopneumothorax doivent être traités par la thoracotomie exploratrice immédiate. L'apparition de bulles sub-pleurales dans le radiogramme du poumon constitue également une indication pour leur exision. L'occlusion sous courant d'eau peut être employée dans les cas de pneumothorax initial et de degré réduit. Cette méthode s'applique aussi comme mesure d'urgence

chez les patients affectés de pneumothorax sous tension ou bilatéraux. Le traitement par aspiration à la sonde ou l'attente ont rarement procuré la prompte et complète réexpansion du poumon. Dans ces dernières conditions, l'incidence des récidives est élevée. L'expectative a donné une mortalité de 5,7% dans cette série de malades. La thoracotomie et l'exérèse des bulles sub pleurales rompues assurent l'éradication de la lésion et produisent une prompte et complète réexpansion du poumon. Cette forme de traitement a prévenu de nouvelles récidives.

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TUBERCULOSIS OF THE BREAST[1]

A Review with the Additional Presentation of Ten Cases

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INTRODUCTION

The present report is concerned with a review of the subject of tuberculosis of the breast and the presentation of the experience with 10 cases at the New York Hospital-Cornell Medical Center. The occasion which immediately prompted the review was experience with a patient with a history of previous tuberculosis of the breast.

The patient was a 37-year old woman who had had her right breast removed for tuberculosis at the Memorial Hospital in 1950. No other focus of tuberculosis could be demonstrated at that time and she was given 1 gm. of dihydrostreptomycin intramuscularly daily for two weeks postoperatively (a dose which by present standards is too small). She developed tuberculous meningitis two months following mastectomy and was placed on streptomycin intramuscularly and intrathecally. In the course of her convalescence she became blind, and a diagnosis of optic atrophy was made. This patient first came under the writer's care in 1952 when she was in her sixth pregnancy. In the past eighteen years she had a normal full-term delivery in 1934, a premature delivery in 1945, and three spontaneous abortions in 1940, 1946, and 1948. She was delivered of a normal infant weighing 3,200 gm. in April, 1953, and is now in her sixth month of gestation.

In reviewing this patient's history, the writer realized that he had never seen a case of tuberculosis of the breast in a pregnant woman. A review of all of the full-term and premature deliveries in patients with pulmonary or extrapulmonary tuberculosis at the Woman's Clinic of the New York Hospital revealed that from 1932 through December, 1954, 743 pregnant patients with tuberculosis had been delivered. In none of these patients was tuberculosis of the breast present. In searching the records of the surgical service of the same hospital, 10 cases were found, from 1932 to the present time, in which the patients had been operated on for tuberculosis of the breast. An analysis of these 10 cases is the basis of this report.

INCIDENCE

Since the first description of tuberculosis of the breast in 1829 by Sir Astley Cooper (1), who recorded "scrofulous swellings in the bosom" of young women with enlargement of the cervical glands, approximately 520 cases have been reported. In 1931 Morgen (2) collected 439 cases, and subsequently approximately 75 additional cases have been described. At the New York Hospital in the past five years, 2 patients were found to have tuberculosis of the breast in

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2,141 operations on the female breast. There were 556 malignant and 1,585 benign lesions removed during this period. This incidence of approximately one per 1,000 corresponds to that at the Memorial Hospital. However, the incidence of tuberculosis of the breast reported from other institutions ranges from 0.5 to 1.4 per cent (3–9).

It is of interest to note that tuberculous mastitis is not usually associated with the more advanced stage of pulmonary tuberculosis. The writer has observed only 2 patients with tuberculosis of the breast, neither of whom was a pregnant woman, among several thousand patients with far advanced tuberculosis at Sea View and Triboro hospitals in New York. Raw (10) states that in thirty years' experience in a tuberculosis sanatorium, where he saw more than 10,000 patients, only seven cases of tuberculous mastitis were recorded, and three of these were in men.

AGE

The majority of the cases reported in the literature have been described as occurring in the child-bearing age. In this group, from twenty to forty years of age, tuberculosis is most prevalent. In the present series of 10 cases, 3 patients were more than sixty years of age (the oldest was seventy-two), 3 patients were between forty-five and fifty years, and 4 patients were between the ages of twenty and forty-four years. Deaver and McFarland (4) reported that 70 per cent of their patients were from twenty to fifty years of age. Schipley and Spencer (9) state that the average age of their patients was forty-four years.

PATHOGENESIS

In the past, large numbers of cases of breast tuberculosis have been incorrectly classified as primary. This is an unfortunate misnomer, for most of these lesions were undoubtedly secondary to a focus elsewhere in the body and reached the breast by one of several routes which will be described. While it is true that the breast may be the only organ showing clinical evidence of tuberculosis, absolute proof that the primary disease began in the breast is possible only if a complete autopsy shows the tuberculous lesion to be limited to the breast or the breast lesion to be the oldest tuberculous process in the body. No proof was offered in these so-called primary cases other than that the patient was in apparently good health and had no previous history of active tuberculous infection. It is the writer's opinion that primary tuberculosis of the breast is extremely uncommon and should be reserved for the rare instance in which direct inoculation of the breast occurs from an infected needle or instrument. Such a case was described by Deaver and McFarland; a nurse who fell on an infected instrument developed tuberculosis of her breast directly thereafter (4).

The cutaneous covering of the breast or nipple may become involved in a tuberculous process in the same manner as lupus of other areas is produced. Cases of mammary tuberculosis have been reported in which the organism gained entrance to the tissue through abrasions in the skin and nipples. Whether infection can occur through the lactiferous ducts is difficult to prove in the late stages of the infection when the interstitial tissues are involved.

The secondary type, in which the patient has a history of tuberculosis or presents herself with active tuberculosis in the adjacent tissues, is undoubtedly the usual mode of involvement of the breast by tuberculosis. The following routes have been described:

Lymphatic spread: The breast is infected by retrograde lymphatic extension from tuberculous nodes in the axilla. This is the most usual route, although occasionally the cervical or mediastinal nodes may be the focus from which spread to the breast occurs. Involvement of the axillary nodes was noted in 50 to 75 per cent of the cases in the literature (2, 4, 11, 12) and was present in 7 of the 10 patients of the present series.

Spread from contiguous structures: The active focus of tuberculosis may be in the rib, sternum, or costochondral junction. Direct spread of tuberculosis from the underlying chest wall has also been described (13, 14). Two patients who developed tuberculosis of the breast tissue following a tuberculous empyema necessitatis were seen by the writer at Sea View Hospital. The breast may also be infected in operations on the lung for pulmonary tuberculosis if the incision is extended into breast tissue and a wound infection ensues.

Ductal infection: Tuberculous infection from the lactiferous ducts may initiate a focus of tuberculosis in the alveoli of the breasts, but this route is extremely rare.

Hematogenous spread: Tuberculous infection of the breast from the blood stream has seldom been reported. Nagashima (15) was unable to demonstrate miliary tuberculosis in the breast in thirty-four autopsies on patients who had tuberculosis of the internal organs or widespread miliary tuberculosis. Not only did he examine several blocks of sections from each breast microscopically, but he also did cultures and guinea pig inoculations of breast tissue without finding tuberculosis in any instance.

PREDISPOSING FACTORS

Parity: Anspach (16) found that 47.5 per cent of forty patients with tuberculosis of the breast had borne children, and Deaver and McFarland (4) reported that 51 per cent of forty-five patients had had a previous pregnancy. The present writer has not observed tuberculosis of the breast during or immediately following pregnancy in more than 1,200 patients who had pulmonary or extrapulmonary tuberculosis. It would appear that the mammary changes incidental to pregnancy do not predispose to tuberculous infection of the breast.

Lactation: Lactation has been termed an important predisposing cause of tuberculous mastitis (17). This is difficult to prove. That a woman nurses her child many years before developing tuberculosis of the breast is not sufficient reason for ascribing the tuberculous infection to lactation. Morgen (2) found that only 185 of 439 patients with tuberculosis of the breast had children. Of the 185 women who had delivered children, many did not breast feed. Since both single women and non-nursing mothers have developed tuberculosis of the breast in larger proportions than have patients who have nursed their children, it seems reasonable to infer that lactation does not play a prominent part in the development of tuberculosis of the breast. It should be recorded, however, that

in the Woman's Clinic patients with tuberculosis are not allowed to breast feed their infants.

Trauma: Traumatic injury of the breast is rarely followed by tuberculous infection. In five of the forty-eight so-called primary cases reported by Deaver and McFarland, trauma seemed influential in the development of the tuberculous process. Morgen states that 7 per cent of the 439 cases he collected from the literature gave a history of trauma. The view has been advanced that injury to an organ may produce an area of decreased resistance, as has been described in bones and joints, and thus a latent focus of infection in the breast or in adjacent structures may be incited to activity by the trauma.

Antecedent mastitis: In some of the series of tuberculous mastitis reported, a history of suppurative mastitis antedated the tuberculous process from one to thirty-seven years. Deaver quotes Scudder's series of 53 cases in which half the patients had borne children and 18.8 per cent had had a previous attack of mastitis. An acute pyogenic mastitis may immediately precede, or occur coincidently with, a tuberculous infection of the breast.

PATHOLOGY

Tuberculosis of the breast is usually unilateral and in only a few instances have both breasts been involved. This is in contrast to the usual tendency of the tubercle bacillus to affect each of bilateral organs (lung, kidney, fallopian tube, epididymis). It confirms the view that tuberculous mastitis rarely results from a hematogenous infection. The following types of tuberculous mastitis have been described:

Nodular (discrete, disseminated, or confluent): The majority of cases of tuberculous mastitis are of the discrete, nodular variety. The skin is usually unbroken and the mass unattached to the underlying structures. Depending on the course of the disease, the mass may break down, causing fluctuation which varies with the extent of liquefaction and secondary changes in the nodule. Progression of the disease may result in abscess formation, with the production of one or more sinuses. In the disseminated or confluent type the nodules may be diffused throughout the breast, forming a large confluent mass or several masses. These may break down and form multiple sinuses.

Sclerosing type: The sclerosing type of tuberculous mastitis is most commonly seen in older patients. It progresses slowly and, although small abscesses may form, they are usually masked by a dense sclerotic reaction. The breast may become shriveled and the nipple retracted, so that this type of mammary tuberculosis is often mistaken for carcinoma. Caseation or sinus formation is not a common feature of this type of breast tuberculosis.

Atypical types: In the type described as *tuberculous mastitis obliterans*, the inflammation is confined to the walls of the milk ducts and peri-acinous connective tissue, with slight involvement of the interlobar stroma. Marked epithelial proliferation occurs in the ducts, followed by necrosis and periductal fibrosis, leading to obliteration of the lumen. Another rare type of breast tuberculosis is that in which an intraglandular cold abscess is formed.

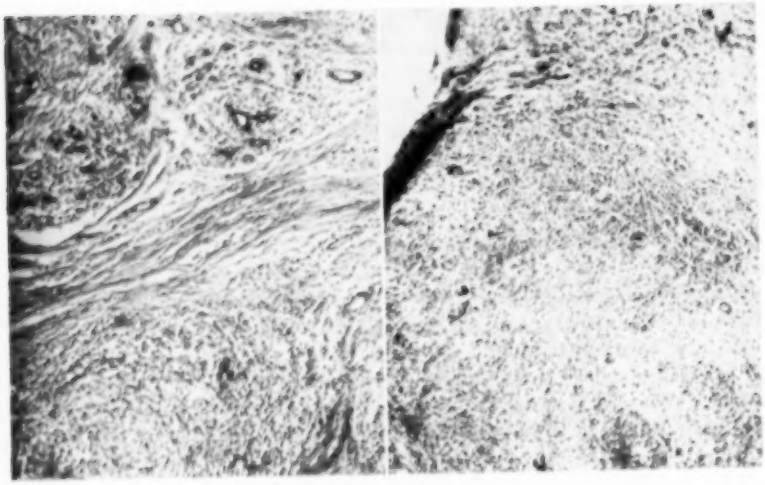

FIG. 1 (Left). Microscopic section revealing normal elements of the breast in the upper portion and a tubercle in the lower portion.

FIG. 2 (Right). Microscopic section showing a tuberculous abscess of the breast in which numerous giant cells are seen.

Microscopic Appearance

The microscopic appearance of tuberculosis of the breast is similar to that of tuberculosis in other organs. In figure 1 are revealed normal elements of breast, namely acini, connective tissue, and fat in the upper portion of the section. There is a diffuse infiltration with lymphocytes. In the lower portion of the section a tubercle may be seen which contains epithelioid cells and giant cells, and which is surrounded by a zone of lymphocytes.

When only a biopsy or aspiration of a breast lesion is taken, there may be no evidence of normal breast structures on microscopic examination. Shown in figure 2 is a tuberculous abscess of the breast in which an area of caseation is present, as are numerous giant cells. A small area of stratified squamous epithelium (skin) may be seen in the upper left area of the section.

BACTERIOLOGY

M. tuberculosis has been cultured from breast lesions and has also been found on direct smear. In seven of Deaver's seventy-nine cases, tubercle bacilli were recognized on microscopy of direct stained smears. The diagnosis of tuberculosis has also been made by aspirating material from a breast abscess and inoculating it into guinea pigs. In the past, tubercle bacilli of bovine origin have frequently been identified but, with the improved technique of bacteriologic examinations and with the almost universal practice of pasteurizing milk, tubercle bacilli of human origin will probably be more frequently found. It would appear to be a

worthwhile procedure, particularly in those instances of breast abscess in which only an incision and drainage of the lesion are done and no material is removed for microscopic examination, to do a complete bacteriologic examination for tubercle bacilli, including direct smears, cultures, and guinea pig inoculations.

SYMPTOMS

The most frequent initial symptom of tuberculous mastitis is a painless lump. This has been noted in 65 to 75 per cent of the cases reported. The lump gradually increases in size and may or may not become fixed to the skin. Pain is an infrequent initial symptom but may occur later in the disease in the course of abscess formation. Axillary lymph node enlargement is present in 50 to 75 per cent of the patients and frequently may precede the development of the breast swelling. The disease progresses, if untreated, for a period of several months and an abscess may form which either ruptures or is incised, resulting in the formation of draining sinuses. Occasionally, secondary infection with pyogenic organisms produces a more acute course.

In the sclerosing type, the mass becomes hard and fixed to the skin and deeper structures. Depending on its proximity to the central portion of the breast, the process may cause retraction of the nipple.

SIGNS

Tuberculous mastitis may occur in patients who appear in apparent good health despite the fact that unrecognized foci of tuberculosis may be present in other parts of the body. The upper outer quadrant of the breast is most frequently involved, due to the proximity of the axillary lymph nodes from which retrograde lymphatic extension occurs. However, any portion of the breast may be affected, including the central portion under the nipple. A nontender, nodular mass is usually the first sign detected. Redness and tenderness of the skin overlying the mass are signs that develop if the condition progresses to abscess formation. Deaver and MacFarland have described retraction of the nipple in approximately one-third of seventy-nine cases. Discharge from the nipple is not a frequent sign. Fistulae occur in approximately one-third of the cases (4, 11).

The appearance of the breast depends on the type and extent of the underlying disease. In the nodular type, no enlargement of the breast may be discernible, whereas, in the confluent type, the involved breast may appear enlarged. Fluctuation with abscess formation and redness of the skin is found in patients who present themselves late in the course of the disease. In the sclerosing type the breast may appear smaller, and retraction of the skin and fixation to the deeper structures may be apparent.

ROENTGENOGRAPHIC STUDY OF THE BREAST

Roentgenographic study of the tuberculous breast has been described by Moore and Hamilton (18). A deficiency of glandular tissue is noted, the striae are intact, and the mass appears relatively localized. In contrast to these findings, the

breast harboring malignant disease shows a destruction of the striae, a less dense opacity, and a mass which appears to extend beyond the confines of the gland (12). The presence of sinus tracts, calcification, or caseation in a roentgenogram of the breast is said to be pathognomonic of tuberculosis.

LYMPH NODE INVOLVEMENT

The axillary lymph nodes on the same side as the involved breast are enlarged in 50 to 75 per cent of the cases. In the majority of cases the nodes are tuberculous and, as mentioned above, in some instances the tuberculous adenitis may precede the development of the breast lesion. However, secondary infection of the tuberculous lesion of the breast with pyogenic organisms may result in a simple hyperplastic adenitis of a nontuberculous nature.

DIFFERENTIAL DIAGNOSIS

Because of the rarity of tuberculosis of the breast, its similarity to other conditions which involve the breast, and the fact that it is seldom correctly diagnosed preoperatively, the several other conditions for which tuberculosis is mistaken will be briefly mentioned.

Before tuberculous mastitis has progressed to the stage of sinus formation and when only a lump is palpable in the breast, it may be mistaken for carcinoma, a fibro-epithelial type of tumor, chronic cystic mastitis, pyogenic mastitis, gumma, sarcoma, and actinomycosis. Carcinoma has been most frequently diagnosed in patients with tuberculous mastitis, particularly in the sclerosing type, but also in the nodular type when fixation of the skin and axillary adenopathy are present. As a rule, tuberculous mastitis occurs at an earlier age than carcinoma and progresses more rapidly. Some of the tuberculous lesions are quite large when the patient first presents herself and, in the absence of extensive metastasis in such a patient, the lesion is more apt to be tuberculosis than carcinoma. Discharge from the nipple, when present, is more often purulent in tuberculosis, whereas it is serous or bloody in carcinoma.

Benign fibro-epithelial tumors (adenoma, fibro-adenoma, fibroma) are usually freely movable, circumscribed, present no skin involvement, and are not associated with axillary lymph node enlargement. *Chronic cystic mastitis* is usually of longer duration than tuberculosis. It is frequently bilateral and is more pronounced several days before the onset of menstruation. Skin involvement and sinus formation rarely occur with chronic cystic mastitis. *Pyogenic mastitis* runs a more acute course than tuberculous mastitis. In some instances tuberculous mastitis may result in abscess formation and secondary infection with other microorganisms. Bacteriologic examination of the abscess material may occasionally reveal tubercle bacilli. After incision and drainage, the pyogenic abscess usually heals promptly, whereas the tuberculous abscess may form chronic sinuses. Serologic tests for syphilis will aid in the diagnosis of a *gumma of the breast*, although a positive serologic reaction is not to be taken as definite evidence that the breast lesion is not tuberculous since patients with tuberculosis may also have syphilis. Darkfield examination of suspicious lesions for *T.*

pallidum may prove useful in such cases. *Actinomycosis* may resemble tuberculous mastitis in so far as it forms multiple sinuses. The presence of sulphur granules and the identification of the ray fungus will reveal the correct diagnosis.

It is apparent that the differential diagnosis of tuberculosis from other breast lesions is not simple. Biopsy for histologic study and complete bacteriologic examination afford the only dependable means of accurate diagnosis.

TREATMENT

In addition to the local treatment of tuberculous mastitis, such general measures as proper rest, diet, and a careful search for other foci of tuberculosis are of importance. Before the availability of antimicrobial therapy the treatment was usually surgical, the procedures varying from curettage, cauterization, injection of the sinuses and cavities, aspiration and incision of abscess, to radical mastectomy and axillary lymph gland dissection. Roentgen-ray therapy and heliotherapy were also used, occasionally alone, but also as a pre- or postoperative adjuvant. Adair (19) has stated, "Frequently a local segmental removal of the breast is not curative, because the sinus tracts are tortuous and the operator is quite apt to leave behind an area of tuberculous mastitis in which another abscess will develop later." Many operators have advocated simple mastectomy in all cases and believe that this is the most conservative therapy.

An electrosurgical technique employing a transverse incision has been described by Moore and Hamilton (18) for the treatment of tuberculosis of the breast. The advantages claimed for this method over the more orthodox scalpel technique are that it prevents local recurrence and the incisions are made more quickly and with less trauma. The electrosurgical technique is not only hemostatic but also seals tissue spaces, eliminating the danger of dissemination and the opportunity for formation of a focus of infection in the severed tissues.

Although only a few cases have been treated preoperatively with antimicrobial therapy, and none have received adequate doses by present standards, the writer believes that surgical treatment of tuberculous mastitis will become modified by the use of the newer antimicrobial drugs. A few patients have received streptomycin-PAS postoperatively and the results, as far as healing of the operative site is concerned, were good. It would appear, judging from the reaction of tuberculosis to antimicrobial therapy in other organs, that the best treatment for tuberculous mastitis would be a preoperative course of antimicrobial drugs, to include streptomycin or isoniazid along with PAS. In the presence of sinus tracts and abscess formation, isoniazid, because of its ability to penetrate deeper into tuberculous cavities than streptomycin, should be the preferred drug. In the absence of any active tuberculous lesion elsewhere in the body, streptomycin may be used in conjunction with it. If other tuberculous foci are present and prolonged therapy for them is contemplated, PAS may be used with the isoniazid, and streptomycin may be kept in reserve for future complications. The antimicrobial agents should be given for a period of at least six months postoperatively, depending on the presence or absence of other tuberculous foci.

The type of operative procedure to be performed depends on the extent of the

lesion. All tuberculous foci in the breast should be removed as well as all tuberculous axillary lymph nodes. This may require simple mastectomy or local removal of the infected section of the breast with a margin of normal tissue. The danger of local recurrence will be greatly reduced by the employment of antimicrobial agents preoperatively, and better wound healing can be expected. In the event that tuberculous mastitis is first discovered postoperatively by microscopic examination, a course of antimicrobial drugs is indicated. If all foci of tuberculosis have been removed, no further operative measures are necessary. However, if inadequate procedures, such as incision and drainage, or curettage of the abscess cavity have been done, the patient should be reoperated upon after three months of antimicrobial therapy and the tuberculous foci in the breast removed.

In tables 1 to 3 a compilation of the data from the 10 patients with tuberculosis of the breast who were operated upon at the New York Hospital from 1932 through 1954 are given.

There were 8 white and 2 non-white patients. The youngest woman was 20 and the oldest 72; the average age was 45.2 years. Seven patients were married and 3 were single. Four patients had borne children; 6 had never been pregnant. In only 2 of the 10 patients was a family history of tuberculosis elicited and only one of the 10 patients was aware of a past history of tuberculosis. Chest roentgenograms were negative for tuberculosis in 8 patients, showed a healed hilar node in one patient, and active pulmonary tuberculosis in another. The general health of 5 of the 10 patients on admission to the hospital was good. One patient was admitted in heart failure, and the lump in the breast was noted on routine physical examination; one patient had been treated for syphilis; one patient had diabetes; one patient had a concomitant carcinoma of the breast. The one patient with bilateral pulmonary tuberculosis and tuberculosis of the spine was in poor condition.

In none of the patients was a history of lactation obtained which immediately preceded the onset of symptoms. Irritation by the cast in the patient who had tuberculosis of the spine and injury from being struck by a handle bar of a bicycle three and one-half years previously were the only instances where trauma was remotely implicated in the development of tuberculosis of the breast in these 10 patients.

The right breast was involved in 6 patients, the left in 4. The duration of symptoms varied from no symptoms in the 2 patients in whom a lump in the breast was palpated on routine physical examination to two and one-half years. Six patients had symptoms for one to four weeks before seeking medical attention; one patient had symptoms for three months; and one for two and one-half years. The presenting complaint was a lump in the breast in 4 patients, a lump associated with pain in an additional 4 patients, and no symptoms in 2 patients. Seven of the 10 patients complained of pain in the breast in the course of their illness. The mass was located in the upper outer quadrant in 6 patients, in the lower middle portion of the breast in 2 patients, in the inner upper quadrant in one patient, and under the nipple in one patient.

TABLE 1

NEW YORK HOSPITAL SERIES OF TUBERCULOSIS OF THE BREAST

Number	Patient	Year	Age	Color	Marital Status	Parity	Family History	Chest Roentgenogram	Past History of Tuberculosis	General Health	Lactation	Trauma
1	A. P.	1933	32	White	Married	2	Negative	Negative	No	Good	0	0
2	D. D.	1935	36	White	Single	0	Negative	Negative	No	Good	0	0
3	R. E.	1936	28	Non-white	Single	0	Negative	Healed hilar glands	No	Good	0	3½ years previously*
4	M. G.	1938	63	White	Married	3	Negative	Negative	No	Heart failure	0	0
5	M. W.	1943	46	White	Married	4	Negative	Pulmonary tuberculosis	Bilateral pulmonary tuberculosis four years; tuberculosis of the spine	Poor	0	yes†
6	M. K.	1944	49	White	Married	0	Negative	Negative	No	Good	0	0
7	M. N.	1945	72	White	Married	1	Son died of tuberculosis	Negative	No	Carcinoma of breast‡	0	0
8	M. B.	1945	20	White	Single	0	Negative	Negative	No	Good	0	0
9	D. S.§	1950	61	White	Married	0	Tuberculosis	Negative	No	Diabetes; fever for three weeks	0	0
10	W. R.	1951	45	Non-white	Married	0	Negative	Negative	No	Syphilis, treated in 1947	0	0

* Breast struck by bicycle handle three and a half years ago; developed lump and draining sinuses one year later.

† Associated lump in breast with wearing plaster jacket and tight girdle which caused pressure against breast.

‡ Operated on for carcinoma of breast two months previously. At secondary radical operation tuberculosis found; no additional carcinoma.

§ Vaginal hysterectomy done two years later revealed tuberculous abscess of uterus.

TABLE 2

NEW YORK HOSPITAL SERIES OF TUBERCULOSIS OF THE BREAST

Number	Side Involved	Duration Symptoms	Initial Symptoms	Tumor Mass		Pain	Condition of Skin	Condition of Nipple		Fistulae or Sinus Formation
				Size	Location			Retraction	Discharge	
1	Right	3 months	Lump	3 x 6 cm.	Upper outer quadrant	Yes	Normal	0	0	0
2	Left	2 weeks	Lump and pain	4 x 5 cm.	Inner upper quadrant	Yes	Red	Raised	0	0
3	Right	2½ years	Lump and pain	2 x 3 cm.	Under nipple	Yes	Ulcerated	Retracted	0	+
4	Left	0	Lump found on routine physical examination	4 x 4 cm.	Upper outer quadrant	No	Normal	0	0	0
5	Left	1 month	Lump and pain	3 x 4 cm.	Lower middle quadrant	Yes	Red	0	0	+
6	Right	3 weeks	Lump	2 x 3 cm.	Upper outer quadrant	Yes	Normal	0	0	0
7	Right	0	Lump found on routine physical examination	2 x 3 cm.	Upper outer quadrant	No	Normal	0	0	0
8	Left	3 weeks	Lump	6 x 10 cm.	Lower middle quadrant	Yes	Red	Retracted	Bloody	0
9	Right	3 weeks	Lump and pain	3 x 6 cm.	Upper outer quadrant	Yes	Red	0	0	0
10	Right	1 week	Lump	3 x 6 cm.	Upper outer quadrant	No	Normal	0	0	0

TABLE 3

NEW YORK HOSPITAL SERIES OF TUBERCULOSIS OF THE BREAST

Number	Enlargement of Axillary Nodes	Treatment	Result	Pathology (Microscopic)	Follow Up
1	0	Local excision	Good	Tuberculous mastitis	5 years
2	0	Incision and drainage; local incision	Good	Tuberculous mastitis; abscess	2 years
3	0	Local excision; packed for two months	Good	Tuberculous mastitis	12 years
4	Yes	Local excision; lymph node dissection	Good	Tuberculous mastitis	3 years
5	Yes	Local excision; packing	Good	Tuberculous mastitis; abscess	Died after one year of pulmonary tuberculosis
6	Yes	Local excision; axillary lymph node dissection	Good	Tuberculous mastitis; lymphadenitis	2 months
7	Yes	Radical mastectomy; lymph node dissection	Good	Miliary tuberculosis of breast and axillary node	7 years
8	Yes	Incision and drainage; local excision of abscess wall	Healed slowly; good	Tuberculous mastitis; abscess	10 years
9	Yes	Incision and drainage; excision of two-thirds of abscess wall; packing	Healed slowly; good	Tuberculous mastitis; abscess	3 years
10	Yes	Local excision; streptomycin and PAS for two weeks	Good	Tuberculous mastitis	1½ years

The skin was normal in appearance in 5 patients, reddened over the lump in 4 patients, and ulcerated in one patient. The nipple was retracted in 2 patients, raised in one, and appeared normal in 7. In one patient a bloody discharge could be expressed from the nipple. Two patients had sinus tracts leading to the skin from the tumor mass. The axillary lymph nodes were enlarged in 7 patients and not palpable in 3.

Treatment consisted of local excision with or without drainage in 6 patients. In those patients who were packed, several months elapsed before the wound healed. Two patients had local excision of the mass plus axillary lymph node dissection, and one patient had a radical mastectomy with axillary lymph node dissection. One patient had local excision followed by streptomycin-PAS postoperatively. The end results were good in all 10 patients, although the wounds that were packed healed slowly. Four of the 10 patients had abscess formation in the breast.

SUMMARY

The incidence of tuberculosis of the breast compared to other mammary lesions at the New York Hospital in the past five years was 0.1 per cent. The incidence reported in the literature is from 0.5 to 1.4 per cent. Tuberculosis of the breast is most prevalent between twenty and fifty years of age.

The breast is usually infected with tuberculosis by lymphatic spread from the axillary lymph nodes. It may also be involved by extension from contiguous structures in the chest wall.

Parity, lactation, and trauma are not significant predisposing factors in the development of mammary tuberculosis. The most usual pathologic type of breast tuberculosis is the nodular. The sclerosing type and atypical forms occur less frequently.

A painless lump in the breast is the most frequent initial symptom of breast tuberculosis. Any portion of the breast may be involved by tuberculosis. The most common site is the upper outer quadrant. A nontender nodular mass is usually the first sign detectable in tuberculosis of the breast. Abscess formation and pain may occur later in the course of the disease. The presence of fistulae, retraction of the nipple, and enlarged axillary lymph glands is highly suggestive of tuberculosis.

Roentgenographic study of the breast may occasionally aid in the diagnosis of tuberculosis of the breast. Complete bacteriologic examination of material removed or aspirated from the breast may be of help in diagnosing tuberculous mastitis.

Treatment for tuberculous mastitis should include a pre- and postoperative course of antituberculous drugs and local excision of all tuberculous tissue including axillary lymph nodes, if present. If the diagnosis of tuberculosis is made postoperatively, the patient should receive a course of antituberculous drugs and be reoperated upon if all tuberculous tissue has not been initially removed.

SUMARIO

Tuberculosis de la Mama

La incidencia de la tuberculosis mamaria en relación con otras lesiones mamarias en el Hospital New York en los últimos cinco años fué de 0.1 por ciento. La incidencia mencionada en la literatura varía de 0.5 a 1.4 por ciento. La tuberculosis de la mama es más frecuente a la edad de veinte a cincuenta años.

La mama suele infectarse con tuberculosis por difusión linfática desde los ganglios axilares. Puede también ser invadida por propagación de los tejidos contiguos de la pared torácica.

El parto, la lactancia y el traumatismo no constituyen importantes factores predisponentes en la producción de la tuberculosis mamaria. La forma histopatológica más habitual de esa tuberculosis es la nodular. La forma esclerosante y las formas atípicas se presentan con menos frecuencia.

Un bulto indoloro en la mama es el más frecuente síntoma inicial de la tuberculosis mamaria. Cualquiera parte del órgano puede ser invadida por la tuberculosis. El sitio más común es el cuadrante externosuperior. Una tumefacción nodular insensible suele ser el primer signo descubrible. Más tarde en la evolución puede haber formación de absceso y

dolor. La presencia de fístulas, retracción del pezón e hipertrofia de los ganglios linfáticos de la axila es muy indicativa de tuberculosis.

El estudio radiográfico de la mama puede a veces ser de ayuda en el diagnóstico de la tuberculosis mamaria. El completo examen bacteriológico del material extraído o aspirado de la mama puede ayudar a diagnosticar la mastitis tuberculosa.

El tratamiento de la mastitis tuberculosa debe comprender un régimen pre- y post-operatorio de drogas antituberculosas y la excisión total de todo tejido tuberculoso, incluso los ganglios linfáticos axilares, si lo hay. Si se hace postoperatoriamente el diagnóstico de tuberculosis, el enfermo recibirá una serie de drogas antituberculosas y será reoperado si no se extirpó antes todo el tejido tuberculoso.

RESUME

Tuberculose du sein

L'incidence de la tuberculose du sein comparée à d'autres lésions mammaires au New York Hospital durant les cinq dernières années était 0,1 pour 100. L'incidence rapportée dans la littérature varie entre 0,5 et 1,4 pour 100. C'est entre vingt et cinquante ans que la tuberculose du sein est prédominante.

Habituellement l'infection tuberculeuse du sein se fait par la voie lymphatique issue des ganglions axillaires. Le sein peut aussi être intéressé par extension des lésions thoraciques contiguës.

La grossesse, la lactation et le traumatisme ne sont pas des facteurs prédisposants appréciables dans le développement de la tuberculose mammaire. Le type pathologique le plus commun dans la tuberculose du sein est la forme nodulaire. Les formes sclérosantes ou atypiques sont moins fréquentes.

Une grosseur indolore dans le sein constitue le symptôme initial le plus fréquent de la tuberculose du sein. N'importe quel segment du sein peut être atteint par la tuberculose. Le siège le plus commun est le quart supéro-externe. La masse nodulaire non sensible à la pression est habituellement le premier signe décelable dans la tuberculose du sein. La formation d'abcès et la douleur peuvent survenir plus tard au cours de l'affection. La présence de fistule, de rétraction du mamelon et l'hypertrophie des ganglions lymphatiques axillaires suggèrent fortement le diagnostic de tuberculose.

L'étude radiographique du sein peut parfois contribuer au diagnostic de la tuberculose du sein. L'examen bactériologique complet des prélèvements obtenus par excision ou aspiration peut faciliter le diagnostic de mastite tuberculeuse.

Le traitement de la mastite tuberculeuse doit comprendre une cure pré et post opératoire avec des agents tuberculostatiques ainsi que l'excision locale de tous les tissus tuberculisés, y compris les lésions des ganglions lymphatiques axillaires. Dans le cas où le diagnostic de tuberculose est post-opératoire, la malade devra être soumise à la médication antituberculeuse et être réopérée ensuite si tous les tissus tuberculisés n'ont pas été excisés initialement.

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Case Reports

TUBERCULOUS MENINGITIS: USE OF CORTICOTROPIN AS AN ADJUNCT TO CHEMOTHERAPY[1,2]

Case Report and Review of Literature

J. RICHARD JOHNSON

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INTRODUCTION

Although there is ample evidence that both corticotropin and cortisone may exert a deleterious effect upon tuberculosis in animals and men, accumulated experience in more than a hundred tuberculous patients has demonstrated no adverse effect upon the disease when use of these hormones is accompanied by effective chemotherapy (1). This fact has encouraged further study of the effects of cortisone and corticotropin on tuberculosis by diminishing inflammation and fibrosis and by decreasing toxicity. The use of these drugs in the presence of tuberculosis may be required for the treatment of serious complicating conditions, e.g., severe drug reactions (2), collagen diseases (3), postoperative collapse, and probably otherwise uncontrollable arthritis or asthma. Cortisone would also be necessary for a patient with Addison's disease with or without tuberculosis (4). In the treatment of tuberculosis itself, the use of the hormones is generally accepted in more critically ill patients with tuberculous meningitis (5) and perhaps in the patient with pulmonary or miliary disease who succumbs to toxicity early in the course of antimicrobial therapy (6).

Recently, wider use of corticotropin or cortisone in the treatment of certain forms of tuberculosis has been reported. These forms include tuberculous meningitis (7, 8, 9), pleurisy with effusion (10), and even pulmonary tuberculosis (2, 11). Generally, good results are achieved and never with aggravation. Cortisone and corticotropin are also of value as frankly symptomatic treatment of the terminal tuberculous patient (12). In allergic reactions to streptomycin, corticotropin given concurrently for only four to six weeks may permit continuation of the antimicrobial therapy without difficulty (2). It should be kept in mind, however, that, even though concurrent antimicrobial drugs may prevent corticotropin and cortisone from affecting tuberculosis adversely, animal experiments indicate that these hormones can impair the effectiveness of the drugs (13, 14).

Tuberculous meningitis probably affords the best indication in tuberculosis for the use of corticotropin or cortisone. The marked symptomatology, high mortality, and high incidence of disabling sequelae in this disease warrant the use of these potent agents if they are of value. Although survival rates have improved from 25 per cent or less with streptomycin alone and 50 per cent with streptomycin and para-aminosalicylic acid (PAS) to the present 75 per cent or better with the addition of isoniazid, tuberculous meningitis is still a disease which kills or maims too many of its victims.

In 1951, Kinsell (6) recommended the use of corticotropin in tuberculous meningitis

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[2] Presented, in part, at the Fourteenth Conference of the Veterans Administration, Army, and Navy, on Chemotherapy of Tuberculosis, Atlanta, Georgia, February 7, 1955.

along with the usual antimicrobial treatment and described dramatic cures in one moribund patient and in one patient who was declining rapidly. Shane and Riley (7) had a patient who became moribund when a cerebrospinal fluid block developed. The addition of cortisone to the streptomycin and PAS used in that case resulted in prompt clinical remission and relief of the block. Shane and Riley also described six more patients who who were treated with combined cortisone-antimicrobial therapy, with good results in four of them. Barnard (8) treated thirty patients with adjunctive oral and intrathecal cortisone or hydrocortisone for two to four months. Cerebrospinal fluid protein and cells decreased more rapidly and the patients experienced more rapid symptomatic improvement with such treatment, but the writer believed there was no ultimate advantage in using these steroids along with the usual antimicrobials. No evidence of adverse effects on associated pulmonary tuberculosis was seen; in fact, it appeared there was more rapid improvement than usual. Bulkeley (9) treated thirty-one cases of tuberculous meningitis with corticotropin plus streptomycin, isoniazid, and PAS, using the hormone from one to five weeks. He believed it was responsible for bringing patients out of coma rapidly, usually in less than one week. He also noted a fall of protein and cells in the cerebrospinal fluid followed by a slight rise for a few weeks after withdrawal of the corticotropin. Veterans Administration protocols for the treatment of tuberculous meningitis have included the addition of corticotropin or cortisone to the antimicrobial regimens of desperately ill patients. The Committee on Therapy of the American Trudeau Society (5) acknowledges that such combined therapy may be of value for cerebrospinal fluid block.

There is no real evidence to date that tuberculous disease in man is aggravated by corticotropin or cortisone when effective antituberculous drugs are being employed. The rationale for the use of such combined therapy in tuberculous meningitis is based on one or more of the following effects of these hormones:

Modification of the inflammatory exudate and granulation tissue in the meninges with decrease in mechanical blockage and pressure and decrease in the amount of such tissue to be organized into fibrotic scar tissue

The so-called "vitalizing" or detoxifying effect to enable the patient to survive the critical first two to three weeks when most deaths occur, thus allowing time for the antimicrobials to act

The suppression of tuberculous arteritis as demonstrated in animal experiments by Ebert (15), thus decreasing vascular damage which is said to cause the neurologic sequelae

The often postulated, but never proved, effect of permitting better permeation of the antimicrobials into the inflammatory tissue to reach the bacilli

CASE REPORT

For seven months a 47-year-old Negro male had complained of intermittent frontal and right-sided headaches which finally culminated in an attack of syncope. For the next two days he suffered an excruciating headache, fever, and somnolence, and was admitted to the hospital.

The temperature was 101°F., pulse 100 per minute and the blood pressure 150 systolic, 116 diastolic in mm. of mercury. He was somnolent, confused, photophobic, and complained bitterly of head pain. No abnormalities were noted on physical examination.

Because of increase in symptoms and questionably reddened ear drums, he was placed on streptomycin, 1 gm., and penicillin, 400,000 units daily, the day after admission. The following day a lumbar puncture was performed because of progression of symptoms and the appearance of a markedly stiff neck.

Cerebrospinal fluid pressure was 450 ml. of water; there were 250 lymphocytes per cu.

mm. The sugar determination was 35 mg. per 100 ml.; the protein, 220 mg. per 100 ml., and the chlorides were 105 mEq. per liter. Smears were negative for bacteria, fungi, and acid-fast bacilli, and all cultures of this and subsequent specimens were negative *except* for a growth of tubercle bacilli on culture from this first specimen six weeks later.

A chest roentgenogram revealed only a small nodule high in the right apex. Other laboratory studies were not remarkable.

The patient was transferred to the tuberculosis service, and treatment was begun with 1 gm. of streptomycin, 1 gm. of dihydrostreptomycin, and 500 mg. of isoniazid daily. Calcium PAS was started seven days later. Except for a reduction of dosage to 1 gm. of streptomycin twice weekly and 350 mg. of isoniazid daily after four months, this therapy has continued without interruption.

After four days of treatment the patient's disorientation, headache, and stiff neck were unrelieved, and there was progressive increase of fever and cerebrospinal fluid abnormality (figure 1). At this point aqueous corticotropin in a dosage of 25 mg. every six hours was added. Complete remission of symptoms and fever followed. After ten days of such combined treatment, the corticotropin was discontinued during a three-day period. Within three days the clinical manifestations promptly recurred. The hormone was reinstituted and again there was complete clinical remission, this time in twenty-four hours. The corticotropin was continued in full dosage for two weeks and then withdrawn gradually over the next two and a half weeks without adversely affecting the patient. He has no sequelae and has been completely asymptomatic for the ensuing five months except for an attack of thrombophlebitis which occurred apparently coincidentally at the time the corticotropin was finally discontinued.

Total eosinophils dropped from 44 to zero, rose to 66 during the withdrawal of corticotropin; fell again with the second course, and eventually rose again after hormone therapy was discontinued.

The 17-ketosteroid excretion rose from 13 to 22 mg. in twenty-four hours and did not fall abruptly during the period when corticotropin was discontinued, thus helping to rule out temporary pituitary-adrenal depression as a factor in the clinical relapse.

Tuberculin tests are interesting in that there was only a minor decrease in size during the first course of corticotropin and a minor increase during the period of omission of the

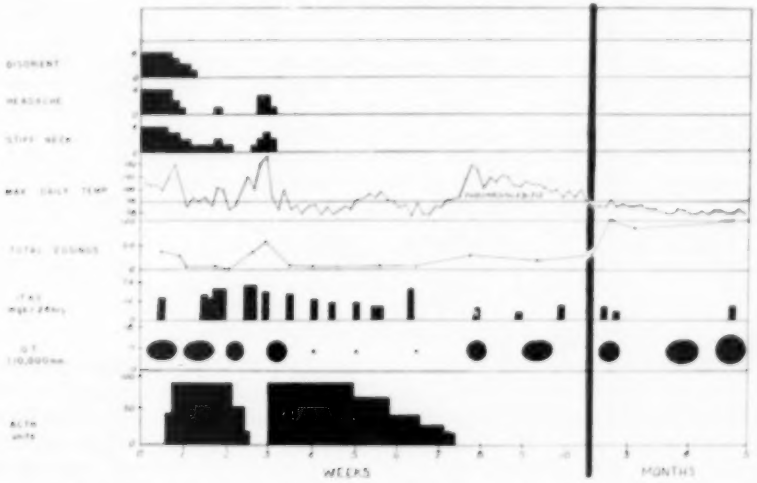

FIG. 1. Relation of corticotropin to clinical and laboratory findings.

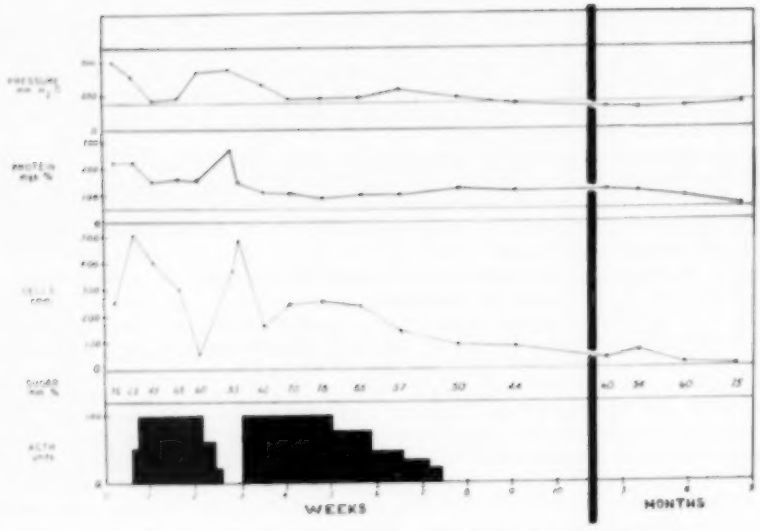

FIG. 2. Relation of corticotropin to cerebrospinal fluid changes.

drug, with disappearance during the second course and subsequent return to baseline levels after discontinuation of corticotropin. Old Tuberculin (OT) tests with 1:100 concentration produced less erythema during corticotropin treatment, but the induration did not decrease until the last two weeks of treatment and then only from 20 x 20 to 10 x 20 mm.

During the first four days of treatment before corticotropin was added, the cerebrospinal fluid pressure and protein remained at high levels (figure 2.). The cells increased from 250 to 500 per cu. mm. and the sugar dropped from 35 to 23 mg. per 100 ml. The sugar rose to normal levels the first week of corticotropin treatment and remained normal thereafter.

When corticotropin was first administered, the cells dropped to 60 per cu. mm. in ten days, rose to 480 when the drug was withdrawn, responded again to the second course, and declined to normal during the next four months. Both protein and pressures fell during the first course of corticotropin, rose during withdrawal, and then dropped to normal values during the next few months.

During the period of corticotropin administration, the patient received penicillin or oxytetracycline (Terramycin®), 800 mg. sodium diet, and potassium chloride supplements. Daily weights and weekly fasting blood sugars were of value. Electrolyte values remained constant. Two weeks of Dicumerol® therapy were required for the thrombophlebitis. The writer wishes to emphasize the fact that streptomycin, isoniazid, and PAS were used throughout the illness without interruption and will be continued for eighteen months or more.

COMMENT

More than 70 reported cases of tuberculous meningitis have been treated with corticotropin or cortisone with at least symptomatic improvement in most cases and with apparently dramatic reversal of the disease process in some. The nature of the disease makes it difficult to prove the value of these hormones by a controlled concurrent treatment study. For this reason it is believed that the technique of interrupted corticotropin treatment employed in the present case is of interest, since the patient served as his own

control. More cases are needed, but certainly the present study demonstrates the marked effect of corticotropin on both clinical and cerebrospinal fluid manifestations.

Unfortunately, the use of corticotropin or cortisone is not simple. The physician who uses these drugs assumes an increased responsibility for even closer clinical and laboratory observation of the patient. In addition to the theoretical possibilities of aggravating the patient's tuberculosis or or decreasing the effectiveness of his antimicrobial therapy, there is always the hazard of causing fluid retention, hypokalemia, diabetes mellitus, peptic ulcer, pyogenic infections (including superimposed pyogenic meningitis (9)), osteoporosis, and post-treatment pituitary and adrenal depression.

To prevent or detect these complications, the following are advisable, at least during the period of high hormone dosage: routine chest roentgenograms, sputum examinations, and urinalyses; low salt intake and daily weights when possible; administration of 2 gm. of potassium chloride three times daily; serum electrolyte and fasting blood sugar determinations once or twice weekly; antacids for any minor gastrointestinal distress; penicillin or a wide-spectrum antimicrobial drug. If treatment is continued more than two months, testosterone propionate and estrogens may be added to prevent osteoporosis. When corticotropin or cortisone are discontinued, the dosages should first be reduced gradually. After corticotropin is discontinued, recovery of the pituitary may be expected in a few days to a week; but, when cortisone is used for a prolonged period, adrenal depression may last for months and any incidental severe stress, such as an operation or a complicating infection, may precipitate relative adrenal insufficiency and require supplemental cortisone administration.

If cortisone is used, neither 17-ketosteroid nor total eosinophilic counts are helpful in evaluating the effect on the patient. With corticotropin therapy both 17-ketosteroid and total eosinophilic counts are of value in measuring the highly variable adrenal response. No response would indicate prompt substitution of cortisone; very high responses (a urinary output of 50 to 100 mg. 17-ketosteroids per twenty-four hours) would suggest dosage reductions. Other indications of response to hormone therapy are rise of the total leukocyte count with a fall in lymphocytes, a rise of fasting blood sugar, a tendency to accumulate fluid, and often a rounding of the patient's face. The most pertinent measures of response, however, will be rapid symptomatic improvement, lysis of fever, and improvement in cerebrospinal fluid cells, protein, sugar, and pressure abnormalities.

Short courses of treatment aimed at accomplishing symptomatic recovery and affording emergency relief have ranged from one to five weeks (6, 9). Long-term therapy, used mostly at low dosage and in an effort to suppress the inexorable process of meningeal and vascular fibrosis, has been continued for three to twelve months (7, 8). The following intermediate course might be suggested: At first full dosages of corticotropin or cortisone may be continued until the patient is asymptomatic or for a maximum period of two or three weeks. Dosages may then be reduced to approximately half the initial level in two steps from seven to ten days. With the reduced dosage, these hormones may be expected to exert strong anti-inflammatory action with a minimum of complications.

This reduced dosage should be maintained so long as there is progressive improvement in the patient's clinical condition or cerebrospinal fluid abnormalities. When the patient becomes asymptomatic or stabilized, the dosages may be discontinued in two or three more steps. Naturally, various complications would necessitate more rapid discontinuation of the cortisone or corticotropin and relapse would require temporary return to higher dosage levels. Intravenous hydrocortisone (compound F) may be necessary as emergency treatment at the outset in desperately ill patients and, of course, hydrocortisone may be substituted for cortisone throughout the regimen.

It is urged that frequent tuberculin tests be made during cortisone or corticotropin therapy, for this evaluation may be the most significant indication of the degree of inhibition of the specific inflammatory response. Marked depression or complete obliteration of the first-injection Old Tuberculin test, 1:10,000, is to be expected; but, should this occur also with the 1:100 strength, the dose of corticotropin or cortisone probably should be reduced. The aim of hormone therapy should be to inhibit the inflammatory response partially but not completely.

An analysis of experience in animals reveals that the contradictory reports of whether or not cortisone and corticotropin exerted deleterious effects on tuberculosis or on antimicrobial treatment of tuberculosis might well be explained by the presence or absence of tuberculin hypersensitivity at the time of hormone treatment (14, 16, 17). In addition, two recent studies with tuberculosis in rabbits revealed that cortisone (18) and high doses of corticotropin (19) adversely affected the animals chiefly by eliminating the protection of partial immunization. Both of these facts imply the importance of preserving some measure of tuberculin hypersensitivity during adrenal steroid therapy.

On the basis of experiments on animals, some investigators have concluded that cortisone may be more hazardous than corticotropin. However, since a given amount of corticotropin produces relatively less compound F in animals than it does in man, equivalent dosages of the two drugs in man will not be equivalent when applied to animals. Certainly, if a given amount of cortisone will adversely affect tuberculosis, it follows that corticotropin will also, providing enough is given to produce the equivalent amount of compound F.

There are, however, certain advantages in the use of corticotropin. With it, 17-ketosteroid and total eosinophilic counts afford a measure of the effect; the level of adrenal steroid response is limited, perhaps to more nearly physiologic levels, by the capacity of the patient's adrenals to respond; and, finally, the resultant pituitary depression is a more transient and less serious phenomenon than the adrenal atrophy resulting from cortisone. A theoretical advantage of aqueous corticotropin every six hours as compared with intramuscular cortisone is that the effects are intermittent, thus not affording continuous suppression of the inflammatory response or of the pituitary-adrenal axis. This does not hold when oral cortisone is used. On the other hand, when adrenal response to corticotropin is inadequate or when an absolute emergency exists, cortisone or hydrocortisone will be preferable, and at times indispensable.

SUMMARY

The beneficial effect of corticotropin on the clinical and cerebrospinal fluid manifestations of a patient with tuberculous meningitis was demonstrated by an interrupted course of corticotropin as an adjunct to treatment with streptomycin, isoniazid, and para-aminosalicylic acid (PAS). The rationale for the use of corticotropin and cortisone in the treatment of tuberculous meningitis was presented in detail. The demonstrated value of corticotropin on both clinical and cerebrospinal fluid manifestations tentatively, at least, suggests its early use in tuberculous meningitis as an adjunct to chemotherapy unless the patient's initial condition is good and unless he shows a prompt response to the usual therapy.

SUMARIO

Meningitis Tuberculosa: Empleo de la Córticotropina como Coadyuvante de la Quimioterapia. Presentación de un Caso y Repaso de la Literatura

El efecto beneficioso de la córticotropina sobre las manifestaciones clínicas y céfalo-raquídeas en un sujeto que padecía de meningitis tuberculosa quedó demostrado por una

serie continua de córticotropina como coadyuvante del tratamiento con estreptomicina, isoniacida y ácido para-aminosalicílico (PAS).

Repásase el empleo de la córticotropina y la cortisona en el tratamiento de la meningitis tuberculosa, según aparece en la literatura.

El valor demostrado de la córticotropina sobre las manifestaciones tanto clínicas cuanto céfalorraquídeas sugiere, a lo menos tentativamente, su uso temprano en la meningitis tuberculosa como coadyuvante de la quimioterapia a menos que el estado inicial del enfermo sea bueno y a menos que muestre respuesta rápida a la terapéutica habitual.

Resume

Méningite tuberculeuse: Emploi de la corticotrophine comme adjuvant à la chimiothérapie—observation d'un cas et compte-rendu de la littérature

L'action favorable de la corticotrophine sur les manifestations cliniques et le liquide céphalo-rachidien chez un malade atteint de méningite tuberculeuse fut démontrée par une cure intermittente de corticotrophine, comme adjuvant au traitement par la streptomycine, l'isoniazide et l'acide para-aminosalicylique (PAS).

L'auteur passe en revue l'emploi de la corticotrophine et de la cortisone dans le traitement de la méningite tuberculeuse d'après les rapports publiés.

D'après la démonstration de la valeur de la corticotrophine à la fois sur les manifestations cliniques et le liquide céphalo-rachidien, il est permis de suggérer son emploi précoce, dans la méningite tuberculeuse, comme médication adjuvante à la chimiothérapies auf si l'état initial du malade est bon et à moins qu'il ne réponde rapidement au traitement usuel.

Addendum

The patient was discharged from the hospital in April, 1955, and has remained well to the present time, October, 1955.

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REACTIONS TO PARA-AMINOSALICYLIC ACID[1]

A Case Simulating Infectious Mononucleosis with a Transient Pulmonic Infiltrate

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INTRODUCTION

In the few years since para-aminosalicylic acid (PAS) was added to the physicians' weapons against tuberculosis, a fairly large literature has accumulated, in which have been reported the many untoward effects which have occurred during its use. It has been necessary to differentiate the effects which are toxic, due to intolerance, from the actual allergic sensitivity reactions (1).

Among the sensitivity reactions which have been reported recently are peripheral vascular collapse with a Guillain-Barré syndrome (2, 3); encephalopathy (4); leukemoid reaction (5); schizophreniform psychosis (6); leukopenia and atypical lymphocytosis (7, 8); allergic pneumonitis (9–12); and a clinical state simulating infectious mononucleosis or hepatitis (13–15). The present report deals with a case which presented at different times an allergic pneumonitis and a condition similar to infectious mononucleosis.

CASE REPORT

J. K., a 36-year-old white married male longshoreman, was admitted to the Chest Section of the Medical Service of the Bronx Veterans Administration Hospital on June 2, 1954, with a minimal tuberculous lesion of undetermined activity. There was no history of allergy.

Physical examination was within normal limits. Roentgenographic examination of the chest on admission showed an infiltrate at the level of the fourth intercostal space on the right. Tomographic studies located the infiltrate in the axillary aspect of the anterior division of the right upper lobe. Urinalysis on admission was normal. The leukocyte count was 12,050 per cu. mm., with a normal differential. Sputum was positive for tubercle bacilli on microscopy and culture.

Course in the hospital (figure 1): On the twenty-sixth hospital day the patient was started on a regimen of bed rest, isoniazid, 100 mg. three times daily, and para-aminosalicylic acid (PAS), 4.0 gm. three times daily. The patient did well until the fifty-second hospital day and the twenty-seventh day after commencement of antituberculous therapy. He then complained of nausea and vomiting, cough, and right upper quadrant abdominal pain, which continued the following day. His temperature rose to 102°F. On the fifty-fourth hospital day he had a severe chill with a temperature rise to 105°F. and complained of malaise, tinnitus, headache, nausea, vomiting, cough, generalized aching, and some abdominal discomfort. Physical examination showed slight pharyngeal infection, a few scattered rhonchi in the chest, and moderate distension of the abdomen. A roentgenographic examination of the chest showed no changes. On the following day he had another chill with fever to 105°F., and several small posterior cervical lymph nodes were noted. Since the clinical picture was considered to be compatible with a drug reaction, PAS was discontinued.

During the next two days his temperature rose daily to 104°F., and a diffuse, discrete maculopapular rash involved the trunk, back, and all extremities. On the fifty-sixth hospital day, all medications were stopped except, occasionally, codeine and aspirin. By the fifty-eighth hospital day, there was questionable icterus, the liver was definitely palpable 1 cm. below the right costal margin, and tenderness was noted in the right upper abdominal

[1] From the Veterans Administration Hospital, Bronx, New York, New York.

FIG. 1. Chart showing patient's course in the hospital.

quadrant. The leukocyte count was 9,800 per cu. mm. with 46 per cent lymphocytes, of which 15 were abnormal and suggestive of the Downey cells seen in infectious mononucleosis. The next day, nausea and vomiting subsided completely and the patient's temperature receded to 101°F. By the sixty-first hospital day the rash had started to subside, but there was an increase in lymphadenopathy with appearance of an enlarged suboccipital node and persistent hepatomegaly. The leukocyte count continued to show absolute lymphocytosis, with a large number of abnormal cells and beginning eosinophilia. The urinary urobilinogen was 1:160, and the total serum bilirubin was elevated to 1.7 mg. per 100 ml. There was 11 per cent bromsulfalein retention in forty-five minutes.

A sternal marrow aspiration on the sixty-second hospital day showed an eosinophilia of 20 per cent and was considered compatible with infectious mononucleosis or a drug reaction. The attending hematologist concurred with the impression that the peripheral blood smears were highly suggestive of infectious mononucleosis.

The patient remained relatively asymptomatic for several days, although he continued to have a leukocytosis, with gradual disappearance of the abnormal cells and gradually increasing eosinophilia. Chest roentgenograms taken bi-weekly showed no changes. Serologic tests, including cold agglutinins, *Streptococcus MG*, heterophil antibody, febrile agglutinins, and amebic complement-fixation tests were either negative or were positive only in insignificantly low titers. Four daily blood cultures done at the onset of the reaction were sterile.

On the sixty-sixth hospital day, the patient complained of severe retro-orbital and bilateral frontal headache with nausea, vomiting, and increasing rash with pruritus. His temperature was 101.5°F. Physical examination revealed generalized lymphadenopathy, a liver palpable 2 cm. below the right costal margin, and a spleen palpable 1 to 2 cm. below the left costal margin. The spleen remained palpable for five days. The rash, which involved the entire body including the palms and soles, faded over the next few days. Liver function tests showed a cephalin flocculation of 4-plus, bromsulfalein retention of 31 per cent in forty-five minutes, serum alkaline phosphatase of 10.9 units per 100 ml., and serum bilirubin of 3.3 mg. per 100 ml. (direct/indirect was 1.9/1.4). A repeated heterophil antibody titer was negative.

Review of the chart revealed that the patient had been receiving acetylsalicylic acid and APC® tablets (acetylsalicylic acid, phenacetin, and caffeine) for headaches during this period. A cross-reaction with the salicylates was considered responsible for the recrudescence and they were withheld.

The febrile episode lasted two days. The patient became asymptomatic and both his physical examination and liver function tests returned to normal. On the ninety-second hospital day, he was started on a regimen of desensitization to PAS, starting with 50 mg. per day with gradually increasing doses. By the one-hundred-fourth hospital day, he had received a total of 1.7 gm. during the two-week period.

Para-aminosalicylic acid was discontinued, the last dose having been given at 1 p.m. of the day prior to the febrile reaction. Isoniazid was restarted with a single dose of 100 mg. at 4 p.m. of the same day and the patient received 100 mg. of isoniazid at 7 a.m. the following morning. At 10:30 a.m., the patient had a sudden onset of nausea and vomiting, with a severe shaking chill and violent retching. His temperature rose to 105°F. Examination revealed a mottled skin with a maculopapular erythematous eruption over the abdomen and chest wall. Slight cervical lymphadenopathy was present. The lungs were clear. Subsequently, the patient also complained of severe constricting anterior chest pain and retrobulbar burning. An electrocardiogram was within normal limits. Two blood cultures were sterile.

Corticotropin was administered by intravenous and intramuscular routes, and within twenty-four hours the temperature had returned to normal and the patient was symptomatically improved. The rash became pruritic and more prominent, involving the trunk, extremities, neck, and face, with increased cervical lymphadenopathy and a large epitrochlear lymph node. The liver was palpable 1 cm. below the right costal margin, with a

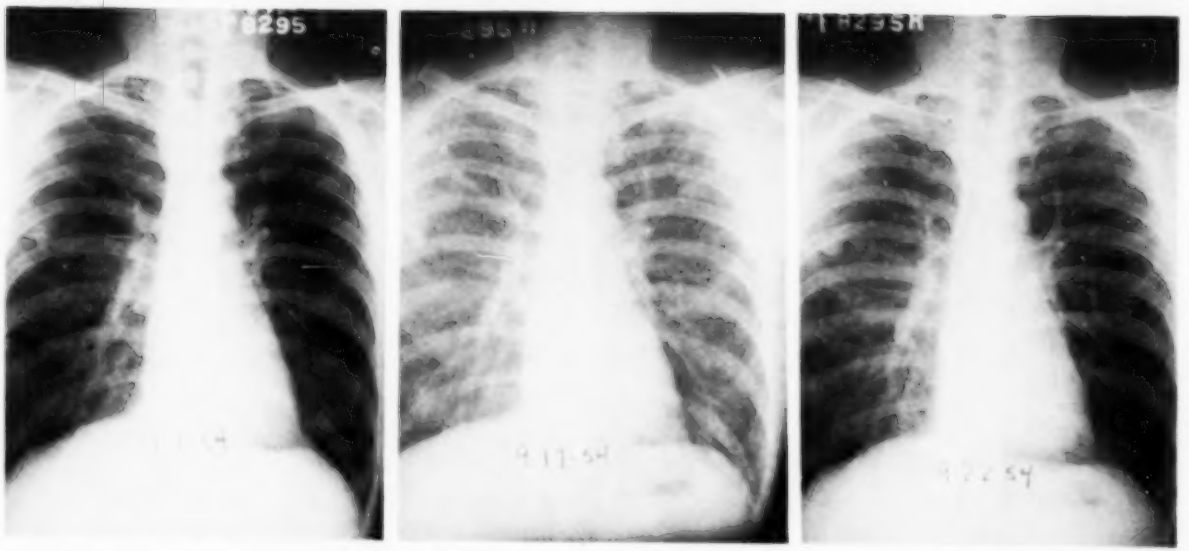

FIG. 2. Roentgenograms of the chest obtained on the ninety-ninth, one hundred and seventh, and one hundred and twelfth hospital days. Note changing right upper lobe infiltrates.

firm, sharp edge. Corticotropin was continued in decreasing amounts through the one-hundred-ninth hospital day and the patient received no other medication.

A roentgenographic examination of the chest on the one-hundred-seventh hospital day showed an increase in the size of the infiltrate in the right upper lobe and a density at the level of the right second anterior interspace overlying the third rib. Within five days, the density at the right second interspace had disappeared and the other infiltrate had returned to its previous size. Leukocyte counts made three to five times weekly showed a marked leukocytosis with an eosinophilia of 3 per cent on the one-hundred-fifth hospital day, gradually increasing to 14 per cent by the one-hundred-twentieth hospital day. There was no lymphocytosis, and liver function tests were normal.

By the one-hundred-thirteenth hospital day the patient was asymptomatic except for occasional pruritus limited to the lower extremities, apparently unrelated to drugs.

Isoniazid was subsequently started in gradually increasing amounts and by the one-hundred-fifty-fifth hospital day the patient was receiving full therapeutic doses of 100 mg. three times daily. He was transferred to the Surgical Service on the one-hundred-ninety-eighth hospital day, and the right upper lobe lesion was removed by wedge resection. The postoperative course was uneventful.

DISCUSSION

Drug reactions have been known to assume many bizarre forms. In the case presented here, two somewhat different manifestations occurred in consecutive reactions to apparently the same medication. The initial picture of hyperpyrexia, nausea, vomiting, abdominal pain, hepatosplenomegaly, lymphadenopathy, rash, gradually increasing eosinophilia, leukocytosis, absolute lymphocytosis, and Downey cells was similar to that reported by Cuthbert (13) and by Lichtenstein and Cannemeyer (15). As noted by Leibowitz (16), the differentiation between drug fever and infectious mononucleosis was aided by the failure of a heterophil antibody response to develop.

The second part of what seemed to be an initial diphasic response appears to have been precipitated by ingestion of acetylsalicylic acid and APC® tablets (acetylsalicylic acid, phenacetin, and caffeine). Warring and Howlett (12) reported that one of their seven cases showed a cross-reaction with salicylates, and Korst (17), in his review, noted that three of seven patients showed a similar hypersensitivity to the salicylates. No attempt was made here to give test doses and the patient later refused scratch testing.

At the time of the patient's second reaction, following a course of desensitization to PAS which was similar to that advocated by Steel (18), the patient had such a marked response that strong countermeasures were indicated. The appearance of the pulmonary infiltrates, transient in nature and without associated physical findings, followed by a gradually increasing eosinophilia, fit within the category of "simple pulmonary eosinophilia" according to the classification of Crofton and associates (19). The roentgenographic, laboratory, and clinical findings were similar to those reported by Warring and Howlett (12), Tuchman (11), and Atwell and Prior (9). It is interesting to speculate just what effect the administration of corticotropin at the height of the reaction had on the delay in appearance of the eosinophilia.

It was not possible to completely rule out isoniazid as the etiologic agent or the possibility of cross-sensitization between isoniazid, the salicylates, and PAS. Isoniazid was being given at the time of onset of both reactions, although it certainly could not be incriminated in the second part of the initial biphasic reaction.

SUMMARY

A case of severe sensitivity reaction to para-aminosalicylic acid (PAS) is presented which was initially manifested by a clinical and laboratory picture closely resembling

infectious mononucleosis with negative heterophil antibody determinations. After a period of desensitization to PAS, the patient had a second severe reaction, with the appearance of transient pulmonary infiltrates and eosinophilia. On treatment with corticotropin, the febrile reaction was controlled within twenty-four hours although the other manifestations were not significantly altered. Cross-reaction with salicylates and possibly with isoniazid is suggested.

Sumario

Reacciones al Acido Para-Aminosalicílico: Cuadro Semejante al de la Enfermedad Sérica y Neumonitis de Löffler

Preséntase un caso de intensa reacción de sensibilidad al ácido para-aminosalicílico (PAS), que se tradujo inicialmente por un cuadro clínico y de laboratorio, que se parecía mucho a la mononucleosis infecciosa con determinaciones negativas de anticuerpos heterófilos. Tras un período de desensibilización al PAS, el enfermo experimentó una segunda reacción grave, con aparición pasajera de infiltrados pulmonares y eosinofilia. Tratada con corticotropina, la reacción febril cedió en término de veinticuatro horas, aunque las otras manifestaciones no se alteraron mayor cosa. Sugiérese una reacción cruzada con salicilatos y posiblemente con isoniacida.

Resume

Réactions à l'acide para-aminosalicylique: Signes analogues à la Maladie du sérum et au Syndrome de Löffler

Observation d'un cas de réaction allergique sévère à l'acide para-aminosalicylique (PAS), dont l'aspect initial en clinique et au laboratoire était très proche de la mononucléose avec détermination des anticorps hétérophiles négative. Après une période de désensibilisation au PAS, le malade a présenté une seconde réaction sévère sous l'aspect d'infiltrats pulmonaires transitoires et d'éosinophilie. La réaction fébrile a été contrôlée dans les vingt-quatre heures par la corticotrophine, mais les autres manifestations n'ont pas été sensiblement modifiées. L'auteur suggère de pratiquer la réaction croisée avec les salicylates et peut-être avec l'isoniazide.

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Notes

THE PYRAZINAMIDE SUSCEPTIBILITY OF ISONIAZID-RESISTANT MUTANTS OF TUBERCLE BACILLI

The activity of pyrazinamide against tuberculous infections in animals and man has been reported by several groups of investigators[1-6]. Several clinical groups[7-9] used pyrazinamide alone; other investigators used it in association with streptomycin or para-aminosalicylic acid (PAS), and reported it to be moderately active in the treatment of human tuberculosis. In comparative clinical studies on patients who received pyrazinamide-isoniazid, however, the regimen was reported to be superior to all other combinations of antituberculous agents[5, 6, 10]. Moreover, the use of these two drugs together was found to approach total eradication of tubercle bacilli in the spleens of experimentally infected mice.[11]

In the clinical and laboratory studies reported, investigators,[5, 10, 12-14] have failed to demonstrate *in vitro* susceptibility of tubercle bacilli to pyrazinamide consistently, with the exception of McDermott and Tompsett.[15] These latter investigators used a medium with lowered pH (5.0 to 5.5, compared with the usual pH of 6.6 to 6.8 in media prepared for the growth of tubercle bacilli).

The present communication reports the observation of a relationship between isoniazid-resistant mutants of tubercle bacilli and their *in vitro* susceptibility to pyrazinamide.

Felsen quadrant plates were prepared containing Middlebrook 7H9 oleic acid-albumin agar medium[16] with one control quadrant containing no drug and with selected isoniazid or pyrazinamide concentrations in the other three quadrants. Pyrazinamide for the medium was prepared by dissolving the drug in distilled water and sterilizing by passage through a Seitz filter. Isoniazid for the medium was prepared by dissolving the drug in distilled water and sterilizing by autoclaving for fifteen minutes at 15 pounds of pressure. The drugs were added aseptically to the sterile medium just prior to pouring the plates. Strains of tubercle bacilli for pyrazinamide susceptibility determinations were subcultured in Middlebrook's 7H9 Tween®-albumin liquid medium[16] and were

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TABLE 1

TYPICAL PYRAZINAMIDE SUSCEPTIBILITY OF ISONIAZID-RESISTANT MUTANTS OF TUBERCLE BACILLI

Strain	Isoniazid Resistance	Pyrazinamide Resistance
	γ per ml.	γ per ml.
H37Rv	0.08	200+
557R	1	200+
604R	2	200+
605R	5	25
313T	8	25
188T	10	10
316T	12	25
MK 8 10	15	25
MK 8 1A	15	200+
Clift*	25	200+

* Denotes name of strain

incubated at 37°C. until grown to a density of between 60 and 77 per cent light transmission as measured at 465 mμ on a Lumatron colorimeter. The suspension was then diluted to 10^{-4} with sterile distilled water; 0.05 ml. of the 10^{-4} dilution (containing approximately 50 to 150 organisms) of the test suspension was inoculated by pipette onto the medium in each quadrant of the Felsen plate. The seeded plates were incubated at 37°C. for three weeks before being examined for growth. When sputum and gastric aspirates were studied, the drug-containing plates were inoculated directly with the decontaminated, concentrated clinical material. After three weeks of incubation the per cent resistant of the test microbial population on the drug-containing quadrants was calculated with reference to the control quadrant containing no drug, assuming the control quadrant to be 100 per cent growth.

The pyrazinamide susceptibility of the H37Rv strain of *M. tuberculosis* was determined by the method described above, and it was observed that 20 to 50 per cent of the

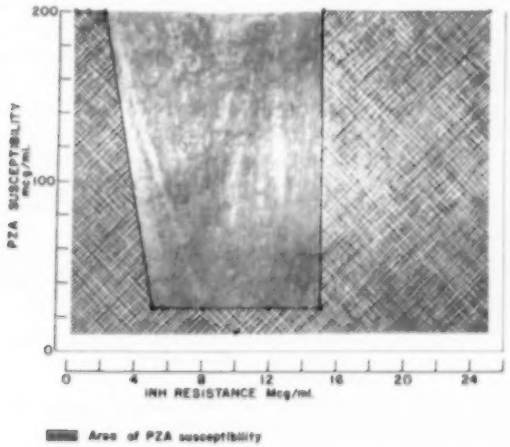

FIG. 1. The pyrazinamide susceptibility of isoniazid-resistant tubercle bacilli.

culturable cells in the suspension were resistant to more than 200 γ of pyrazinamide per ml. of medium. These findings are consistent with the experience of other investigators.

Suspensions of cultures of wild strains of tubercle bacilli recovered from patients and resistant to various concentrations of isoniazid were prepared and tested for their susceptibility to pyrazinamide. In addition, sputum specimens were seeded directly to comparative quadrant plates, containing in one plate selected concentrations of isoniazid and in the second plate selected concentrations of pyrazinamide. Typical data from these studies are presented in table 1 and are plotted in figure 1 as pyrazinamide susceptibility versus isoniazid resistance.

The data presented in figure 1 indicate that a unique relationship exists between resistance to isoniazid and susceptibility to pyrazinamide. Tubercle bacilli resistant to approximately 5 to 15 γ of isoniazid per ml. of medium were completely susceptible to relatively low concentrations of pyrazinamide. Between 0 and 5 γ of isoniazid per ml. of resistance, wild strains of tubercle bacilli were resistant to pyrazinamide concentrations of more than 200 γ per ml. Above approximately 15 γ of isoniazid per ml. resistance, the strains of tubercle bacilli studied were also resistant to pyrazinamide.

* * *

Thus, it becomes apparent that pyrazinamide is primarily effective against mutants of tubercle bacilli which are resistant to isoniazid within specific concentrations, i.e., approximately 5 to 15 γ of isoniazid per ml. This finding is in accord with reports that the combined therapy of tuberculosis with isoniazid-pyrazinamide is superior to other treatment regimens. One may theorize that, as the population of surviving tubercle bacilli in a patient under treatment with isoniazid shifts to specific higher levels of resistance to isoniazid, the tubercle bacilli enter a range of resistance to isoniazid which renders them susceptible to pyrazinamide.

A report[17] which has indicated that pyrazinamide is not effective in patients who have bacilli resistant to isoniazid may perhaps be explained on the basis that all, or the larger proportion, of the isoniazid-resistant mutants recoverable from such patients are resistant to concentrations of isoniazid of 4 γ or less, and are therefore not susceptible to pyrazinamide. Further evidence to support this concept is the fact that the widely used dosage of isoniazid (300 mg. per day) seldom effects serum or plasma concentrations of biologically active isoniazid sufficient to select a population of mutants of tubercle bacilli which fall in the pyrazinamide-susceptible range.[18]

Clinical studies are now in progress at Fitzsimons Army Hospital aimed at investigating the possible chemotherapeutic significance of this phenomenon.

In summary: A unique relationship is described which exists between the isoniazid resistance of mutants of tubercle bacilli and their susceptibility to pyrazinamide. Tubercle bacilli resistant to between approximately 5 and 15 γ of isoniazid per ml. of medium were found peculiarly susceptible to relatively low concentrations of pyrazinamide, i.e., 25 γ of pyrazinamide per ml. This peculiar relationship was found to exist when using an oleic-acid–albumin agar medium at a pH of 6.6.

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May 26, 1955

[17] Moyer, R. E., and Schwartz, W. S.: Tr. Thirteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1954, p. 166.

[18] To be published by Research and Development Unit, Fitzsimons Army Hospital, Denver, Colorado.

VIOMYCIN IN THE RE-TREATMENT OF PULMONARY TUBERCULOSIS

In a recent report, 125 cases of advanced pulmonary tuberculosis treated with viomycin in various Veterans Administration hospitals were analyzed in detail by Tucker.[1] He concluded that viomycin in the dosage of 2 gm. intramuscularly twice weekly was a useful drug in the treatment of tuberculosis, but recommended that it be utilized in association with one or more other effective antimicrobial agents. He found that roentgenographic improvement occurred following viomycin therapy in approximately 50 per cent of the cases and reversal of infectiousness in approximately 30 per cent. The importance of these values is increased by the fact that 97.6 per cent of the 125 patients had received previous antituberculous chemotherapy. Although the specific contribution made by viomycin to these gratifying results could not be evaluated, Tucker believed that the "viomycin was at least helpful."

The significance of the above report was enhanced by the finding that the toxic side reactions of viomycin when given in a dosage of 2 gm. twice weekly were comparatively slight, thus confirming a similar observation made at the Fitzsimons Hospital.[2] In prior clinical reports,[3-6] viomycin was used in higher doses of approximately 40 to 60 mg. per kg. of body weight daily, and had been found moderately effective but quite toxic. The side effects most commonly observed were listed by Hobby[6] as allergic reactions, albuminuria and cylinduria, eosinophilia, disturbances in the electrolyte balance, abnormal renal function (especially in patients with pre-existing renal damage), dizziness, and deafness.

The present writers have treated 29 patients with far advanced pulmonary tuberculosis with viomycin in a dosage of 2 gm. intramuscularly twice weekly. All of the patients had active disease and tubercle bacilli in the sputum despite previous chemotherapy.

Four patients received less than two months of viomycin therapy; two left the hospital against medical advice; one developed side reactions sufficiently severe for the medication to be stopped after only four weeks; and one patient died of pulmonary tuberculosis in his sixth week of viomycin therapy. A fifth patient continued progressively ill in spite of viomycin treatment for slightly more than two months, when therapy was discontinued because of increasing dizziness. This patient died a few weeks later. The remaining 24 patients received viomycin, usually in association with other antituberculous agents, for a minimum of four months and for an average period of approximately eight months. Five of the patients were treated for more than one year. Twenty of these cases were included in Tucker's report, but at that time many had been on viomycin therapy only a short time and the final outcome could not always be predicted.

Of the 24 patients, 62 per cent were excreting streptomycin-resistant tubercle bacilli and, in two instances, the microbial strains were also resistant to para-aminosalicylic acid (PAS). The latter 2 patients were treated with viomycin singly; the remaining 22 patients received additional antimicrobial agents concurrently. Ten received isoniazid; 6, PAS; 4, isoniazid and PAS; one, pyrazinamide and PAS; and one patient received isoniazid, PAS, and oxytetracycline. In the main, the experience with viomycin in the dosage employed was sufficiently unimpressive to stimulate presentation of this report.

[1] Tucker, W. B.: Am. Rev. Tuberc., 1954, *70*, 812.

[2] Pitts, F. W., *et al.*: Tr. Eleventh Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1952, p. 279.

[3] Muschenheim, C.: Tr. Tenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1951, p. 154.

[4] Werner, C. A., *et al.*: Am. Rev. Tuberc., 1951, *63*, 49.

[5] Hobby, G. H.: Tr. Twelfth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1953, p. 300.

Roentgenographic improvement occurred in 8 patients, 7 of whom had received isoniazid in addition to viomycin. Five of these patients had never received isoniazid previously. The eighth patient had received pyrazinamide and PAS, in addition to viomycin. In most of these patients, the roentgenographic improvement was slight but, because of the unfavorable prognosis attached to these cases, any improvement was deemed significant. Among the 8 patients who showed improvement, there were 4 with streptomycin-susceptible and 4 with streptomycin-resistant organisms. All 7 patients who received viomycin and isoniazid had organisms which were susceptible to isoniazid *in vitro*. In Tucker's report, 50 per cent of the patients showed roentgenographic improvement.

In 3 of the 24 patients, tubercle bacilli disappeared from the sputum during viomycin therapy. These 3 were patients who had also received isoniazid for the first time. This experience with respect to reversal of infectiousness compares very unfavorably with the 30 per cent reversal rate found in the larger group analyzed by Tucker. In one patient in the present series, tubercle bacilli disappeared from the sputum on microscopy but not by culture. This patient had been treated with viomycin and isoniazid and had shown roentgenographic improvement, but he left the hospital prematurely, against medical advice. None of the patients treated with viomycin and PAS, or with viomycin alone, demonstrated reversal of infectiousness. It is of interest that one of the latter patients, when later treated with isoniazid, showed marked improvement.

There were some manifestations of drug toxicity in 16 of 27 patients. In only 5, however, was it necessary to discontinue viomycin because of toxicity. Two of these patients had severe aching and malaise. The third, with already decreased renal function, showed a further diminution and also experienced flank pain. The fourth patient developed severe headache and nausea. It was necessary to discontinue viomycin in the fifth patient after only one month's therapy because of severe malaise and a skin rash. The toxic manifestations in the 11 other patients consisted mainly of malaise, aching, slight albuminuria and eosinophilia (up to 19 per cent but usually between 6 and 9 per cent). In 4 of the patients who experienced malaise, it was possible to continue the medication only by diminishing the dose from 2 gm. to 1 gm. twice weekly. Although these patients tolerated the decreased dosage, milder degrees of malaise were often experienced during the day on which the viomycin was received. There was no evidence of electrolyte disturbance in 10 of the patients who were carefully investigated for this abnormality, and no gross eighth-nerve toxicity was noted clinically.

Thus, 59 per cent of the patients experienced some toxic manifestations ascribable to viomycin, as compared with 33 per cent in Tucker's series. It was necessary to discontinue treatment because of toxicity, however, in only 19 per cent of the patients, a value which compares fairly well with the figure of 14 per cent in the larger group.

In summary: Twenty-nine patients were treated with 2 gm. of viomycin twice weekly; 24 of the patients for more than four months and for an average of eight months. Only 33 per cent showed any roentgenographic improvement; only 13 per cent showed reversal of infectiousness (sputum conversion by culture). Fifty-nine per cent of the patients experienced toxic side effects from the drug of varying severity. Treatment was stopped because of drug toxicity in 19 per cent of the group. The most strikingly beneficial results were obtained when the viomycin was given in addition to isoniazid to patients who had never had isoniazid before. From the reports of some observers,[6,7] it is possible that

[6] Cohen, S. S., *et al.*: Tr. Twelfth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army and Navy, 1953, p. 352.

[7] Eckley, G.: Tr. Thirteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army and Navy, 1954, p. 134.

similar good results might have been obtained in these patients with the use of isoniazid alone. It is the consensus at present, however, that multiple-drug regimens should be employed in the treatment of tuberculosis—the so-called "combined therapy." The present experience supports Tucker's opinion that, when treatment with streptomycin or PAS is inadvisable, viomycin may yield good results when given in addition to another effective antimicrobial. At present, isoniazid would be the drug of choice in such a situation.

The difficulty with viomycin lies in the fact that, because of drug toxicity, the dose must be diminished to a point at which the moderate therapeutic effectiveness of the drug is obviously curtailed. The dose of 2 gm. twice weekly of viomycin alone, in the complicated type of case in which its use usually is considered, would appear to show little promise. In a situation in which it appears necessary to employ viomycin alone, the larger doses probably should be employed[8] in spite of the hazard of increased toxicity.

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[8] Schaffield, H. G., *et al.*: Am. Rev. Tubere., 1954, *69*, 530.

THE INTERMITTENT USE OF STREPTOMYCIN AND ISONIAZID SINGLY AND TOGETHER IN MURINE LEPROSY

The prolonged administration of streptomycin and isoniazid, singly and together, in clinical trials against human leprosy has aroused interest in the possible effectiveness of the intermittent administration of these two agents. The satisfactory results achieved in the treatment of tuberculosis with streptomycin administered twice weekly has suggested that this same regimen might be adequate also in the treatment of leprosy. No such data are available, however, with respect to other intermittent streptomycin regimens. Nor are such data available with respect to isoniazid.

In a previous communication from this laboratory, a technique was described whereby the effectiveness of antimicrobial agents might be evaluated in experimental animals on a semiquantitative basis, utilizing *M. leprae murium* as the test organism (Am. Rev. Tuberc., 1954, *69*, 173). This technique appeared suitable also for evaluation of differences in drug regimens. The present report, therefore, will describe results obtained in a series of experiments conducted in this manner, with streptomycin and isoniazid administered singly and together, according to seven different intermittent regimens.

As indicated above, the technique used throughout the present study has been described in detail elsewhere (Am. Rev. Tuberc., 1954, *69*, 173). In all instances mice were infected intraperitoneally with *M. leprae murium*; treatment was initiated on the fourteenth day of infection; the therapeutic results achieved during treatment were measured on the basis of microbial population counts on spleen homogenates from mice sacrificed at various time periods throughout the course of the experiment. Ten animals invariably were used in the preparation of each spleen homogenate.

Streptomycin sulfate and isoniazid were employed in all instances in dosages equivalent to 2.7 and 0.63 mg. of activity, respectively, per mouse per day. Streptomycylidene isonicotinyl hydrazine[1] was administered in a dosage equivalent to 3.9 mg. total weight, i.e., in an amount equivalent to 2.7 mg. of streptomycin activity in combination with 0.63 mg. of isoniazid.

Seven drug regimens were included for evaluation in the study reported herein:

1. A single subcutaneous injection, once weekly (figure 1*a*)
2. A single subcutaneous injection, twice weekly, i.e., on Mondays and Thursdays (figure 1*b*)
3. A single subcutaneous injection, once daily on five consecutive days each week (figure 2*a*)
4. A single subcutaneous injection, once daily on five consecutive days during every other week (figure 2*b*)
5. A single subcutaneous injection, once daily on five consecutive days per week, for two weeks, followed by two weeks of rest; and repeated (figure 2*c*)
6. A single subcutaneous injection, once daily on five consecutive days per week, for four weeks, followed by four weeks of rest; and repeated (figure 3*a*)
7. A single subcutaneous injection, once daily on five consecutive days per week, for four weeks, followed by eight weeks of rest; and repeated (figure 3*b*)

The results obtained throughout this series of experiments are illustrated in figures 1, 2, and 3.

From figures 1*a* and *b*, it is apparent that streptomycin, when administered once or twice weekly in the dosage used herein, was capable of decreasing to a slight extent only the rate of multiplication of *M. leprae murium* within the spleens of infected animals.

[1] Supplied as Streptohydrazid® by Chas. Pfizer & Co., Inc.

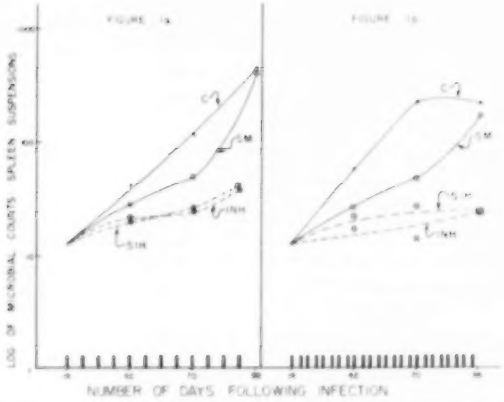

FIG. 1. Populations of *M. leprae murium* in tissue suspensions (T_{50}) of drug treated animals. ▌Black bars indicate days on which treatment was administered. C represents untreated control animals; SM, INH, and SIH represent animals treated with streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine, respectively.

Graph 1*a*. A single subcutaneous injection, once weekly.

Graph 1*b*. A subcutaneous injection, twice weekly.

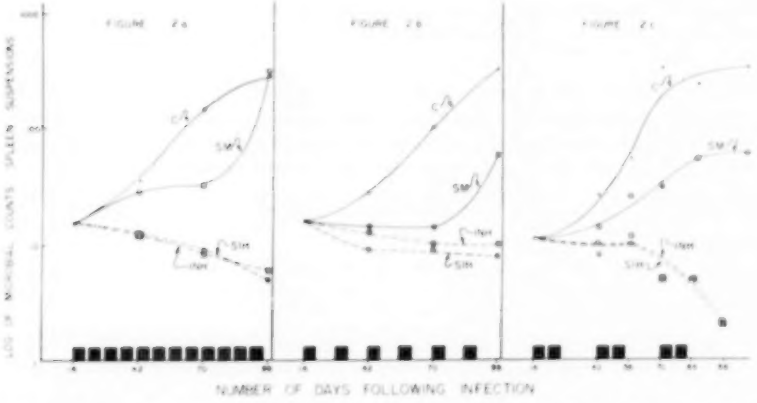

FIG. 2. Populations of *M. leprae murium* in tissue suspensions (T_{50}) of drug treated animals. ▌Black bars indicate days on which treatment was administered. C represents untreated control animals; SM, INH, and SIH represent animals treated with streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine, respectively.

Graph 2*a*. A single subcutaneous injection on each of five consecutive days, each week.

Graph 2*b*. A single subcutaneous injection on each of five consecutive days, every other week.

Graph 2*c*. A single subcutaneous injection on each of five consecutive days per week during the first two weeks of each four-week period.

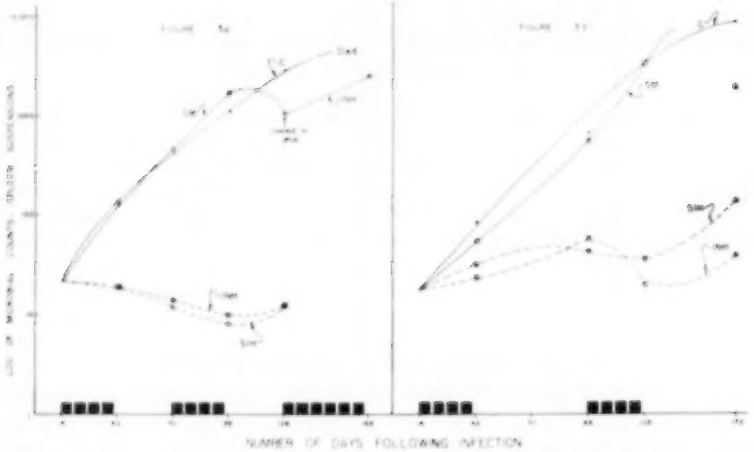

FIG. 3. Populations of *M. leprae murium* in tissue suspensions (T_{24}) of drug-treated animals. ▌ Black bars indicate days on which treatment was administered. C represents untreated control animals; SM, INH, and SIH represent animals treated with streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine, respectively.

Graph 3*a*. A single subcutaneous injection on each of five consecutive days per week during the first four of each eight-week period.

Graph 3*b*. A single subcutaneous injection on each of five consecutive days per week during the first four of each twelve-week period.

Moreover, as illustrated in figures 2*a*, *b*, and *c*, streptomycin likewise was capable of exerting only a slight degree of bacteriostatic action when administered at more frequent intervals. The bacteriostatic effect was temporary and, after approximately seventy days of treatment, there was a sharp rise in the microbial population within the spleens. This rise in the census occurred under treatment with streptomycin and it was assumed, therefore, that it reflected the emergence of *M. leprae murium* cells resistant to the action of this antimicrobial agent.

It is of interest that streptomycin was totally inactive, under the conditions of these experiments, when administered only during the first four weeks of each eight- or twelve-week period (figures 3*a* and *b*).

Isoniazid, like streptomycin, when administered once or twice weekly, decreased to only a slight extent the rate of multiplication of *M. leprae murium* in the spleens of infected mice (figures 1*a* and *b*). When administered more frequently, however, i.e., daily for five consecutive days per week, either every week, every second week, during the first two weeks of each four-week period, or during every other four-week period, it produced a more marked bacteriostatic effect (figures 2*a*, *b*, *c*, and 3*a*). In no instance, however, were all organisms eradicated from the spleens. The census remained at a low level, even though treatment was interrupted in some instances for periods as long as two or four weeks (figures 2*c* and 3*a*).

It is of interest that, when treatment was interrupted for an eight-week period, a small but definite increase in the microbial population within the spleens of the infected animals was detected (figure 3*b*). Re-treatment of these animals with isoniazid after an eight-week

rest period again brought about a decrease in the census, however, and it was apparent that the microorganisms had not lost their susceptibility to this agent. The fact that isoniazid was effective in this instance stands in contrast to the situation illustrated in figure 3*a*. In the latter instance, animals failing to respond to streptomycin therapy were subsequently treated with isoniazid. Probably due to the large number of microbial cells present at the start of isoniazid therapy, no decrease in the population was noted under its influence.

Similar results were obtained in a series of experiments conducted in rats. One-tenth milliliter of a 5 per cent aqueous suspension (containing 0.5 per cent india ink) of rat testes homogenate from animals infected with *M. leprae murium* were inoculated subcutaneously into each of six sites on the back of each rat (Wiersing strain, 250 gm. in weight). Treatment with isoniazid was initiated fifty-one to ninety days after infection, and was administered on five consecutive days each week. In each experiment, a minimum of 5 and generally 10 rats were used for each dosage of each drug tested. Animals were sacrificed at the completion of a seventeen-week course of therapy. Those leproma which had not spontaneously excavated were dissected out, and the leproma from each animal was pooled, weighed, and homogenized. The microbial population within the homogenized tissue was then determined according to the technique commonly employed with mouse spleen suspensions.

In four separate experiments conducted in this manner, isoniazid (5 to 40 mg. per kg. of body weight per day), streptomycin (50 to 100 mg. per kg. per day), iproniazid (20 mg. per kg. per day), and Promin® (50 to 1,000 mg. per kg. per day) failed to diminish appreciably the number of microbial cells present within the leproma homogenates.

In a single experiment, the leproma from 12 rats treated with isoniazid (10 and 20 mg. per kg. of body weight per day), from 12 rats treated with streptomycin (50 and 100 mg. per kg. of body weight per day), and from 6 untreated control animals were homogenized and the resultant suspensions were utilized to infect mice (by the intraperitoneal route). Subsequent determinations of the microbial population within the spleens of these mice at forty-five days after infection indicated that the acid-fast bacilli present in the rat leproma after therapy with isoniazid and with streptomycin were viable.

From these experiments, it was concluded that isoniazid and iproniazid, like streptomycin and Promin®, are incapable of exerting an antimicrobial effect against *M. leprae murium* in rats when large numbers of organisms are present at the start of therapy.

The effects observed with streptomycylidene isonicotinyl hydrazine were comparable to those observed with isoniazid (*See* figures 1, 2, 3).

* * *

The data presented herein represent results obtained in a single experiment only. They are based, however, on observations on more than 1,000 animals. Each population count recorded in figures 1, 2, and 3 represents an average of four counts on a homogenate made from 10 mouse spleens. Thus, it is believed that they probably offer valid information concerning the results that can be achieved on treatment of *M. leprae murium* infections in mice with the doses of streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine used herein.

No correlation may exist between *M. leprae murium* infections in experimental animals and human leprosy, and data obtained in the former infection may not be applicable to the latter. This possibility has been discussed elsewhere (Am. Rev. Tubere., 1954, *69*, 173). If any analogy can be drawn from experiments with *M. leprae murium*, however, it seems probable that more effective results may be achieved in the treatment of leprosy with isoniazid than with streptomycin, and that the combined use of streptomycin with isoniazid may offer no advantage over the use of isoniazid alone. It seems probable, moreover, that, when isoniazid is administered for prolonged periods of time, the treat-

ment period may be interrupted for as long as two to four weeks without loss of effectiveness. The evidence to date suggests, however, that isoniazid may be ineffective when large numbers of organisms are present at the start of therapy.

The possiblity that interrupted regimens, such as certain of those described herein, may be similarly effective against experimental tuberculous infections warrants consideration.

In summary: The effect of seven different dosage regimens of streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine upon the microbial population within the spleens of mice infected with *M. leprae murium* has been described. Data have been presented which confirm the greater effectiveness of isoniazid, as compared with streptomycin, in the control of this infection. Moreover, data have also been presented to show that isoniazid therapy may be interrupted for periods as long as two to four weeks without loss of effectiveness.

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PYRAZINAMIDE-ISONIAZID IN TUBERCULOSIS[1,2]

III. Observations with Reduced Dosage of Pyrazinamide

The results of the treatment of pulmonary tuberculosis with a regimen of pyrazinamide-isoniazid, in which pyrazinamide was administered in a total daily dosage of 50 mg. per kg., have been previously reported from the New York Hospital and the Navajo Medical Service.[3, 4] On the basis of these results, considered together with related studies in animals in this laboratory,[5-7] it was concluded that pyrazinamide-isoniazid exerts antituberculous activity *in vivo* of a sort superior to that of the other antituberculous drugs in current use in either single or multiple drug regimens. It was emphasized that this conclusion rested on three types of evidence, only two of which were provided by the roentgenographic and bacteriologic studies in man. The third type of essential evidence was provided by microbial enumeration studies in experimentally infected mice. This belief that all three types of evidence are necessary has been discussed in the previous reports. It is based largely on the conviction that it is difficult to distinguish among any multiple-drug regimens which contain isoniazid solely by roentgenographic and bacteriologic studies of patients with pulmonary tuberculosis. It was further concluded in the previous studies, however, that pyrazinamide-isoniazid could not be recommended for general use in the dosage studied because of a high incidence of hepatic toxicity.

Accordingly, the effectiveness and relative safety of a lower dose of the pyrazinamide component of the regimen have been studied in patients with pulmonary tuberculosis and the results form the basis of the present report.

A series of 61 patients have been treated on the New York Hospital and Navajo Medical Center services. The total daily dosage of pyrazinamide was 20 to 30 mg. per kg. (hereinafter designated "low-dose pyrazinamide-isoniazid regimen"). As in the high-dose series, the isoniazid was administered in a total daily dosage of 4 to 5 mg. per kg.

Both drugs were given together for twelve to thirteen weeks, with isoniazid alone thereafter.

Of the 61 patients who were started on this regimen, 31 were male and 30 were female. Forty-four of the patients were from the Navajo Medical Service and 17 were from the New York Hospital. All but 5 had received no previous antituberculous therapy. Three of the 5 had received isoniazid for periods of time which varied from ten to twenty-three months.

The patients in this series comprised a prognostically less favorable group than in the

[1] This study was supported in part by grants from the Navajo Tribal Council, the National Tuberculosis Association, made possible by a special bequest from the estate of Sophie Goman; the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service; the Lederle Laboratories division of the American Cyanamide Company, Pearl River, New York; and Charles Pfizer and Co., Inc., Brooklyn, New York.

[2] The pyrazinamide (Lederle Laboratories and Merck & Co., Inc.) and the isoniazid (Nydrazid®, E. R. Squibb & Sons) were generously supplied by the manufacturers.

[3] McDERMOTT, W., *et al.*: Am. Rev. Tuberc., 1954, *69*, 319.

[4] MUSCHENHEIM, C., *et al.*: Am. Rev. Tuberc., 1954, *70*, 743.

[5] McCUNE, R. M., JR., AND TOMPSETT, R.: Tr. Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1954, p. 168.

[6] TOMPSETT, R., *et al.*: Tr. A. Am. Physicians, 1954, p. 67.

[7] McCUNE, R. M., JR., *et al.*: (In manuscript)

original 50 mg. pyrazinamide series. Because of concern about possible hepatic toxicity, only patients with advanced disease considered to have a poor prognosis were admitted to the study. Of the 61 patients, 48 had far advanced and 13 had moderately advanced disease by the 1952 National Tuberculosis Association classification. The methods of study and evaluation used in the present investigation are the same as those used in the high-dose pyrazinamide series.[4]

Of the 61 patients in whom treatment was begun, 52 have completed at least three months of treatment; 42 have completed six months; 19, nine months; and 12, twelve months. Twelve patients were eliminated from the study during the first year of chemotherapy for the following reasons:

In the first quarter, 3 patients were eliminated because of the development of drug toxicity.

In the second quarter, one patient was eliminated for drug toxicity and one by self-discharge.

In the third quarter, 7 patients were eliminated: 3 by transfer to other hospitals, 2 for the performance of surgery, one by self-discharge, and one to receive another experimental antituberculous drug.

Bacteriologic and roentgenographic results in these 12 patients are given only up to the time of their elimination from the study.

As may be seen in table 1, six months after the start of the low-dose pyrazinamide-isoniazid therapy, the roentgenographic results in the 40 patients for whom data were available were designated *substantial improvement* in 32; *no significant change* in 3; and *worse* in no patients.

At nine months data were available for 21 patients and at twelve months for only 13 patients. Accordingly, the six-month assessments have been taken for comparison with the previously reported results with the higher dose of pyrazinamide. There was no essential difference between the two studies in terms of substantial roentgenographic improvement (79 and 80 per cent).

A point of difference between the results in the present low-dose study and the previous study was observed, however, which is not reflected in these over-all assessments. In the present low-dose series, localized and usually temporary increases in the roentgenographic abnormalities were observed in a considerable number of the patients, in contrast to the high-dose series in which this did not occur. The increased roentgenographic abnormalities consisted of extensions of infiltration, which occurred in 7 instances; enlargement of pre-existing cavities, which occurred in 8 instances; and the development of new cavities, which occurred in 3 instances and was associated with an extension of the infiltration in 2 patients. The extensions of infiltration usually occurred early and were of minor degree in all but 2 patients, who had relatively large, although by no means massive, spreads of their disease. In all 7 instances in which spread of disease occurred, it

TABLE 1

PYRAZINAMIDE ISONIAZID ROENTGENOGRAPHIC STATUS

Degree of Roentgenographic Improvement Six Months After the Start of Treatment

Pyrazinamide	Isoniazid	Number of Patients	No Significant Improvement	Substantial Improvement
mg/kg/day	*mg/kg/day*			
50	5	55	13 (24%)	42 (76%)
20-30	5	40	8 (20%)	32 (80%)

TABLE 2

PYRAZINAMIDE-ISONIAZID

Cultures for *M. Tuberculosis*, Sputum, and/or Gastric Aspirate According to Pyrazinamide Dosage

Duration of Treatment	Pyrazinamide Dosage	Negative Cultures (Incidence)	
months	*mg/kg/day*		
3	50	49/53	92%
	20-30	37/52	71%
6	50	48/53	92%
	20-30	28/42	66%

proved to be quickly reversible without any modification of the antimicrobial therapy. The final over-all roentgenographic assessments of the 16 patients who showed an increase in the roentgenographic abnormalities at any time during the course of treatment revealed substantial improvement in 13, slight improvement in 2, and no change in one patient.

It is to be emphasized that the increase in the roentgenographic abnormalities was always reversed or of limited significance, as when enlargement of cavities persisted, and that no patients were worse from the roentgenographic standpoint in the over-all six-month assessment. On the contrary, the majority of the patients were classified as having shown substantial roentgenographic improvement at the most recent evaluation point. The increases of infiltration were for the most part of insignificant degree, occurred early (within the first three months), and cleared rapidly without any change in the chemotherapy. Even the more conspicuous extensions were rapidly reversed. The enlargement of cavities was in no instance associated with a general progression of disease. In 2 of the 3 instances in which new cavities appeared, these subsequently closed. In the third instance, despite the appearance and persistence of a cavity not seen in the pretreatment film, the over-all improvement has been marked.

The relatively large number of these increases in roentgenographic abnormalities in the present study stands in striking contrast to the experience in the previously reported high-dose pyrazinamide-isoniazid series. It is also in contrast to the small number of deteriorations observed in a group of patients treated with isoniazid alone.[8] In the last-named series, only 6 of 47 patients exhibited roentgenographic deterioration of any type or degree and only 2 showed actual spread of disease in the form of extension of infiltration. The fact that extension of infiltration occurred in 7 instances in the present series and that in 6 of the 7 instances this occurred within the first three months of the chemotherapy, i.e., during the period of concurrent administration of pyrazinamide and isoniazid, suggests the possibility that the phenomenon was directly related to the chemotherapy. The evidence is insufficient to permit any inferences whether the phenomenon represented an actual provocative response to the isoniazid or, contrarily, an antagonism of its activity.[9]

[8] DEUSCHLE, K., *et al.*: Am. Rev. Tuberc., 1954, *70*, 228.

[9] During the initial three weeks of the pyrazinamide treatment of mice experimentally infected with a virulent human strain of tubercle bacilli (H37Rv), pyrazinamide regularly antagonizes the effectiveness of isoniazid. This is evidenced by a greater reduction of the splenic microbial populations with isoniazid alone than is seen with pyrazinamide-isoniazid.[8]

All of the patients in the present series had sputum or gastric aspirates which contained *M. tuberculosis* prior to starting treatment. The bacteriologic data obtained from these patients at three and six months after initiation of treatment are presented in table 2. From the standpoint of reversal of infectiousness, these data suggest that the regimen containing 20 to 30 mg. per kg. per day of pyrazinamide was definitely inferior to the 50 mg. per kg. per day regimen. The differences are similar at nine and at twelve months, but the number of patients who have reached these evaluation points is still too small to be conclusive.

Pyrazinamide was discontinued in 4 patients before the end of the projected thirteen weeks because of the occurrence of untoward clinical reactions.

Two patients, both of whom were receiving pyrazinamide at a dosage of 30 mg. per kg. per day, developed definite hepatitis. Both patients were moderately heavy alcohol drinkers but otherwise there was nothing pertinent in their past histories. The first was a fifty-year-old colored female who developed icterus on day 77 of pyrazinamide therapy and had the familiar course of moderately severe hepatitis thereafter, with eventual recovery.

In the present study, the standard liver function tests were not helpful in detecting liver damage until it became clinically apparent. A significant abnormality of the standard liver function tests (bromsulfalein retention of 19.3 per cent) occurred on the sixtieth day of treatment in the second patient, a forty-three-year-old man who had a palpable liver and spleen and mild liver tenderness at this time, but did not develop jaundice or other clinical manifestations of hepatitis.

A third patient developed fever associated with urticaria, eosinophilia, and liver tenderness. This occurred on the eleventh day of therapy. Liver function tests were normal. This was not considered to be a case of toxic hepatitis. The fourth patient, after having completed three months of pyrazinamide and while on isoniazid alone, developed some symptoms suggestive of peripheral neuritis. The neuritis is attributed to the isoniazid.

* * *

Thus, the hope was not realized that reduction of pyrazinamide dosage would result in the maintenance of full pyrazinamide-isoniazid effectiveness while avoiding hepatic toxicity. It is probable, therefore, that, if the powerful antimicrobial effects of this therapy are to enjoy a wider use, simple methods must be devised for the early detection of hepatic damage. Without such methods and without criteria for identifying those especially susceptible to pyrazinamide hepatitis, it seems wise to use the full dose (50 mg. per kg.) and to limit pyrazinamide-isoniazid to use under carefully controlled conditions.

A striking difference exists between the incidence of hepatitis in the published literature, including the present and previous reports from these services,[2, 4, 10] and the incidence of hepatitis in the investigation conducted by the U. S. Public Health Service.[11] It must be recognized, however, that there are certain differences between the type of patients predominant in the U.S.P.H.S. investigation[11] and those in the studies from the present clinics.[3-5]

In the U.S.P.H.S. study, the group was predominantly male and all had received considerable antituberculous chemotherapy at the time pyrazinamide was started. In the studies by the present investigators,[3-5] virtually all of the patients had entirely untreated tuberculosis prior to the pyrazinamide-isoniazid therapy, and the groups have included a

[10] Schwartz, W. S., and Moyer, R. E.: Am. Rev. Tubere., 1954, *70*, 413.

[11] Hepatic toxicity of pyrazinamide, a U.S.P.H.S. cooperative clinical investigation, presented by Dr. George Wilson at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 23, 1955.

substantial number of women. Whether either of these factors is related to increased hepatic susceptibility to pyrazinamide is unknown. At the present time, therefore, the freedom from hepatic toxicity noted in the U.S.P.H.S. study remains unexplained.

In summary: Reduction of the pyrazinamide dosage in the pyrazinamide-isoniazid regimen was nevertheless associated with an incidence of 2 cases of hepatitis among 61 patients treated. Indications were also present that the low pyrazinamide dosages were therapeutically inferior to the higher dosage used in the earlier pyrazinamide-isoniazid studies from these clinics. The therapeutic inferiority was particularly evident at the lowest dosage level of 20 mg. per kg. In view of these findings, it is believed that further clinical investigations of pyrazinamide in tuberculosis might be more advantageously directed to the search for simple methods for the detection of hepatic toxicity than toward manipulation of dosage. An associated finding in the present study was the appearance in relatively high incidence of transient and apparently insignificant increases in the areas of tuberculous pulmonary infiltration during the first ninety days of therapy with the low doses of pyrazinamide and the full dose of isoniazid.

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FURTHER OBSERVATIONS ON THE EFFECT OF CYCLOSERINE ON TUBERCULOSIS IN GUINEA PIGS

In a preliminary report from this laboratory it was shown that, by macroscopic observation, cycloserine appeared to be relatively inactive against tuberculosis in guinea pigs as compared with isoniazid.[1] The purpose of the present paper is to describe histopathologic and bacteriologic studies which have been completed more recently.

The methods used for macroscopic evaluation of the effect of cycloserine[2] on experimental tuberculosis in guinea pigs have been described elsewhere.[1] In addition, histopathologic studies were made of the lung, liver, and spleen of representative animals from each of the three cycloserine-treated groups and also from the control group.[3] A portion of each spleen was also homogenized in 5 ml. of 5 per cent sodium hydroxide containing 0.04 per cent phenol red, allowed to stand for twenty minutes, and was then neutralized with 2 N hydrochloric acid. Three tubes of modified Löwenstein medium[4] were inoculated with 0.3 ml. amounts of each spleen homogenate. After incubation at 37°C. for three weeks, the susceptibility of each of the cultures to cycloserine was determined *in vitro* in a modified Proskauer and Beck liquid medium according to techniques described previously.[5]

Histopathologic examination of 2 of the untreated control animals revealed typical disease with extensive hematogenous dissemination involving the lung, liver, and spleen. The organs of 3 animals from each treatment group exhibited the following findings, according to dosage schedule:

37.5 mg. per kg. per day: The dissemination was less extensive in the lung where the process was that of a tuberculous pneumonia. In two of the three lungs there were no necrotic foci. The lung of the third animal showed small areas of necrosis, but most of it was in the stage of organization. In the livers and spleens the foci were predominately cellular, but some showed central zones of necrosis. The necrosis was usually more advanced in the liver.

75.0 mg. per kg. per day: Two of the lungs exhibited a tuberculous pneumonia in an advanced stage of organization with no central zones of necrosis, while the third lung showed evidence of necrosis. The foci in the livers were cellular and showed no central zones of necrosis. In only one of the spleens was there evidence of necrosis. These findings indicated moderate healing changes.

150.0 mg. per kg. per day: One of the three lungs was free of tuberculous foci and the others exhibited only an occasional isolated focus without central necrosis. The spleens showed no necrotic foci, and in only one liver were there occasional small foci with necrotic centers.

Thus the process here appeared to be one of early and quick healing of an underlying tuberculous process.

Tubercle bacilli were recovered in approximately equal numbers from the spleens of the cycloserine-treated and control guinea pigs. The results of the drug-susceptibility

[1] Patnode, R. A., Hudgins, P. C., and Cummings, M. M.: Am. Rev. Tubere., 1955, *72*, 117.

[2] Kindly supplied by Dr. R. M. Rice, Eli Lilly, and Company.

[3] The writers are indebted to Dr. Oscar Auerbach, Veterans Administration Hospital, East Orange, New Jersey, for the histopathologic examination.

[4] Holm, J. and Lester, V.: Pub. Health Rep., 1947, *62*, 847.

[5] Cummings, M. M., Patnode, R. A., and Hudgins, P. C.: Antibiotics and Chemotherapy, 1955, *5*, 198.

TABLE 1

SUSCEPTIBILITY TO CYCLOSERINE OF TUBERCLE BACILLI REISOLATED FROM CYCLOSERINE-TREATED AND CONTROL GUINEA PIGS

Treatment Schedule of Guinea Pigs	Amount of Growth γ/Ml. of Cycloserine						
	C	2.5	5.0	10	20	40	80
mg/kg/day							
—	++++	+++	++	—	—	—	—
37.5	++++	+++	++	—	—	—	—
75.0	++++	+++	++	—	—	—	—
150.0	++++	+++	++	—	—	—	—

determinations on these cultures are presented in table 1. It will be seen that in no case was there evidence suggesting the emergence of drug-resistant strains. The drug susceptibilities of all of the cultures were essentially identical with that of the parent H37Rv strain which had been used to infect the animals.[5]

* * *

It seems reasonable to conclude that, under comparable experimental conditions, cycloserine is not as effective as isoniazid against experimental tuberculosis in guinea pigs. Although the drug appeared to be rather inactive by macroscopic examination, histopathologic studies of the tissues revealed evidence of healing. This would suggest that treatment of the animals for longer than sixty days might have resulted in a more pronounced therapeutic effect. It should be pointed out, however, that, after fifty-two days of treatment with 20 to 35 mg. of isoniazid per day, tuberculous guinea pigs showed very little evidence of infection.[6] None had gross lesions in the lungs or liver. Microscopic examination of the lung revealed no tuberculous lesions, and only one liver section showed necrosis. Small epithelioid tubercles were found in only two spleens.

It could be suggested that the rather poor response to therapy with cycloserine in guinea pigs might be attributable to the extremely rapid emergence of drug-resistant bacilli. Examination of the results presented in table 1 reveals, however, that the bacilli recovered from both the treated and the control animals showed the same susceptibility to cycloserine as the original infecting culture. It is possible that, as with streptomycin,[7] more prolonged therapy might have resulted in the isolation of resistant strains.

Another factor which might account for the poor response *in vivo* to an antimicrobial which is active *in vitro* is conversion to inactive material in the host tissue. In this regard it seems pertinent to recall that 20 γ of cycloserine per ml. are required to completely inhibit growth of virulent tubercle bacilli *in vitro* in the presence of 5 per cent of guinea pig serum, whereas only 10 γ of drug suffice to completely suppress growth of the same strain in basal medium alone or in basal medium plus 5 per cent of human serum.[5] Still another factor which must be taken into account is the possibility that there may be rapid excretion of drug. The results of studies in this laboratory suggest that blood concentrations of cycloserine in guinea pigs drop rather rapidly following intramuscular injection. At a dosage of 50 mg. per kg., for example, serum concentrations were found to be approximately 32, 21, 9, and 1 γ per ml., at the end of one, two, four, and six hours, respectively.

[5] STEENKEN, W., JR. AND WOLINSKY, E.: Am. Rev. Tuberc., 1952, *65*, 365.

[7] FELDMAN, W. H., KARLSON, A. G. AND HINSHAW, H. C.: Am. Rev. Tuberc., 1947, *56*, 346.

In summary: Under the experimental conditions used in the present study, cycloserine appeared to be relatively inactive against tuberculosis in guinea pigs as compared with isoniazid. Although macroscopic examination showed no marked differences between treated and control animals, histopathologic studies of tissue from treated animals revealed some evidence of healing. This might have become more significant had the animals been treated for a longer period of time.

Cycloserine-resistant tubercle bacilli could not be isolated from the spleens of animals treated for sixty days under any of the three dosage schedules.

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A LYTIC FACTOR ACTING AGAINST MYCOBACTERIUM TUBERCULOSIS

The findings described in this paper are the result of investigations started in July, 1952, and were stimulated by an interesting phenomenon observed in this laboratory.

Among 20 strains of *Mycobacterium tuberculosis*, subcultivated simultaneously, autolysis (the term *autolysis* is being used in this paper for "automatic bacteriolysis") of the developed colonies was noted in 2, strains *B* and *D*. Strain *B* had been obtained from bone-marrow culture and strain *D* from the blood of patients with miliary tuberculosis. Cultures of both of the strains of *M. tuberculosis* had been inoculated on May 23, 1952, and had developed abundant colonies two weeks later. On July 5, 1952, a part of the developed colonies was autolyzed. Microscopically the autolyzed material revealed acid-fast remnants bearing no resemblance to the *M. tuberculosis*.

This phenomenon has been investigated further during the next two years, with *in vitro* and *in vivo* experiments. The results indicate that the lytic factor had all of the properties of a phage acting specifically against *M. tuberculosis*.

In reviewing the literature, the writers were impressed by the scarcity of papers concerning lytic factors against *M. tuberculosis*. In 1925, Richet and Haudiuroy[1] described a bacteriophage-like factor isolated from the sputum of 29 patients; in all instances the sputum also was positive for *M. tuberculosis*. The factor was very resistant to heat and was not destroyed at a temperature of 105°F. for twenty minutes. Steenken[2] reported lysis of old cultures of human tubercle bacilli in a highly acid medium (pH 4 to 5, 8). After a certain period of time, however, new colonies appeared which differed morphologically from the old ones and showed reduced virulence when inoculated into guinea pigs. Steenken did not subcultivate the lytic factor and did not suggest that the lysis observed was due to a bacteriophage. In 1953 Froman and associates,[3] in a preliminary report, and later, in a paper,[4] reported the discovery of four phages derived from saprophytic mycobacteria which were active both against virulent tubercle bacilli and a wide variety of saprophytic acid-fast bacilli. In 1953, Gerundo reported a "phagelike" factor isolated from sputum which likewise contained tubercle bacilli.

The lytic factor described below differs from the phages reported by the above writers in that it acts *specifically* against tubercle bacilli and not against saprophytic mycobacteria. The experiments are as follows:

> Material from the autolyzed cultures of tubercle bacilli was broken into small particles by means of a platinum loop in a test tube containing distilled water. This technique was used only in the first series of experiments. Because the homogeneity of the obtained suspension was unsatisfactory, the following method was used in the other series of experiments.
>
> Material from the autolyzed culture of tubercle bacilli was homogenized with Pyrex glass powder and distilled water in a mortar under aseptic conditions. The obtained suspension was transferred into a sterile tube. After sedimentation of the glass powder, the supernatant fluid was removed and was used either diluted or undiluted. One-tenth of a milliliter of the suspension was added each time to every tube of Lowenstein medium. This was enough to cover the surface of the medium which had been inoculated with a fresh culture of tubercle bacilli.

[1] Richet (fils) and Haudiuroy: Handbuch der Pathogenen Mikro-organismen, Gustav Fischer und Urban & Scharzenberg, Jena, Berlin, und Wien, 1949.

[2] Steenken, W., Jr.: Am. Rev. Tubere., 1938, *38*, 777.

[3] Froman, S., Drake, W., and Bogen, E.: Nat. Tubere. A. Tr., 1953.

[4] Froman, S. Drake, W., and Bogen, E.: Am. J. Pub. Health, 1954, *44*, 1330.

Material from the autolyzed cultures of the two strains of *M. tuberculosis*, *B* and *D*, was mixed with a fresh subculture[6] of *Myobacterium tuberculosis* (strain P^a) and was then inoculated into Löwenstein medium. No growth of tubercle bacilli was observed during a period of one month, while simultaneous control subcultures of strain P^a yielded many colonies of tubercle bacilli after ten days of incubation.

Material from the above negative subcultures of strains *B* and *D* was then mixed with a fresh isolate of the strain *P* of tubercle bacilli and was inoculated into a new series of Löwenstein medium. Again no growth of colonies was observed, while simultaneous control subcultures of strain *P* yielded many colonies of tubercle bacilli after ten days of incubation.

Material from autolyzed cultures of the strain of tubercle bacilli, *D*, in dilutions ranging from 1:20 to 1:20,000, was mixed with the standard strain *P* and inoculated into Löwenstein medium. Mixtures containing dilutions of strain *D*, ranging from 1:20 to 1:200 inhibited the growth of the tubercle bacilli. Mixtures containing dilutions of strain *D* material more than 1:200 showed an abundant growth of tubercle bacilli.

Two guinea pigs (H_{28} and H_{38}) were inoculated with a suspension of autolyzed material from *M. tuberculosis* strains *B* and *D*. Both animals died approximately two months later in profound cachexia. At autopsy no specific lesions for tuberculosis were noted on gross examination.

Material from the autolyzed cultures of strains *D* and *B* was mixed with a suspension of tubercle bacilli (strain *P*) and inoculated into nine tubes of Sauton medium. Material from the mixture was also inoculated into guinea pigs after incubation for periods of: a few hours, and one, three, five, seven, ten, twelve, fifteen, and twenty days, respectively. All animals received the same amount of material, namely 0.5 ml. No growth of tubercle bacilli was observed in the Sauton medium during the above twenty-day period.

The first animal inoculated with autolyzed material which had been incubated with living tubercle bacilli for a few hours died after two and one-half months. Six of the other animals survived this period, although they exhibited profound cachexia and 2 died five to six months after inoculation. Autopsy performed on all of the animals failed to reveal any tuberculous lesions either on gross or histologic examination. There was marked dilatation of the capillaries and fatty infiltration of the liver.[7]

In another experiment, 28 guinea pigs were inoculated with living tubercle bacilli (strain *P*) mixed with dilutions of autolyzed tubercle bacilli strain *D*, ranging from 1:20 to 1:20,000. Three animals inoculated with living tubercle bacilli and the autolyzed material in dilutions of 1:20, 1:500, and 1:1,000 died of tuberculosis within three months. The remaining 25 animals died in profound cachexia two to six months after inoculation with the living bacilli-autolyzed bacilli mixtures. At autopsy of these 25 animals, no tuberculous lesions were revealed either on gross or histologic examination.

These results were unexpected and should probably be attributed to the clumsiness of the technique first used.

An additional 4 guinea pigs were inoculated with living tubercle bacilli (strain *P*) mixed with strain *D* autolysate. All of the animals died of tuberculosis after three and one half months. This was interpreted as an indication that the unknown factor from strain *D* autolysate had lost its activity during the year after its initial appearance on September 16, 1952.

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[6] All subcultures were controlled by animal inoculation of Professor Melanides' laboratory (High Agricultural School).

[6] Strain *P* was isolated from the cerebrospinal fluid of a patient with tuberculous meningitis. As may be seen in the text, experiments on guinea pigs were done only with strain P^a, which proved to be highly pathogenic for the animals.

[7] Autopsies and histologic examinations were performed at the Laboratory of Pathology of Athens University by Professor Eleftheriou.

Second instance of autolysis of cultures of M. tuberculosis: On March 23, 1954, 4 strains of *M. tuberculosis* recently isolated from patients were subcultured on Löwenstein medium. Five days after inoculation, abundant colonies of tubercle bacilli had already developed. On April 13, 1954, two of the subcultures containing 2 different strains of tubercle bacilli were completely autolyzed. Investigation of this second instance of autolysis was carried on in the following manner. The new strains of tubercle bacilli showing autolysis are designated strains *A* and *C*.

Dilutions of autolyzed subcultures *A* or *C*, ranging from 1:10 to 1:100, were mixed with a non-autolyzing strain of tubercle bacilli (strain *X*)[a] and inoculated on Löwenstein media. Growth of tubercle bacilli was inhibited, while simultaneous control subcultures of strain *X* showed abundant growth of tubercle bacilli after eight days. Fifteen days after inoculation, a few colonies of tubercle bacilli developed from mixtures of living tubercle bacilli and the highest dilutions of autolysates *A* and *C*, and after a month all of the subcultures developed colonies of tubercle bacilli.

In another experiment, the standard strain of tubercle bacilli (strain *X*) was inoculated into two series of cultures of Youmans medium. The autolysates from strains *A* and *C* were subsequently added on the first, third, and fifth days, respectively, after inoculation. Two weeks later, all subcultures containing the autolysates were negative, while simultaneous control subcultures of the standard strain of tubercle bacilli (strain *X*) showed abundant growth.

Material from the subculture of the standard strain of tubercle bacilli to which autolysate *A* or *C* had been added on the first day of inoculation (obtained from the previous experiment) was filtered and the filtrate was inoculated on Löwenstein medium, together with the standard strain of tubercle bacilli (strain *X*). A few colonies of tubercle bacilli developed after a week in some of the tubes, while simultaneous control subcultures of strain *X* showed abundant growth of tubercle bacilli. After fifteen days, a few colonies of tubercle bacilli developed in all of the tubes which also contained autolysates *A* or *C*.

The same procedure was repeated with material to which autolysates *A* or *C* had been added on the third day of inoculation (obtained from a previous experiment) with another strain of tubercle bacilli (strain *Y*).[b] The results were as already described for the preceding experiment.

Autolysates *A* and *C* were inoculated into Löwenstein medium together with strains *A* and *C*, respectively, i.e., the strains of tubercle bacilli from which the autolysates were originally derived. Growth was inhibited. Strains *A* and *C*, simultaneously subcultured without the addition of autolysates *A* and *C*, were used as controls. A few colonies developed a week later and abundant growth was observed a month after inoculation.

A mixture of a filtrate from a culture of strain *A* and the strain itself were inoculated into a series of tubes of Löwenstein medium. No growth developed after one month of incubation, while in a series of control tubes abundant colonies of tubercle bacilli were observed.

Two guinea pigs were inoculated with a mixture of strain *A* and the filtrate cultures of strain *A*. One control animal was inoculated with strain *A* only. One month later the control showed tuberculous regional adenitis (puncture of the lymph nodes revealed the presence of tubercle bacilli), while the animals inoculated with the mixtures are still in good health.

[a] Strain *X* was isolated from the cerebrospinal fluid of a patient with tuberculous meningitis.

[b] Strain *Y* was isolated from the cerebrospinal fluid of a patient with tuberculous meningitis.

Third instance of autolysis of M. tuberculosis: On June 18, 1954, 5 strains of *Mycobacterium tuberculosis* were tested for resistance to streptomycin. On June 25, abundant colonies had developed in some of the tubes, including one tube designated *E*. By July 18, the colonies of tubercle bacilli in tube *E* were completely autolyzed. Microscopically the autolyzed material consisted of groups of granules or fragments of distorted bacteria.

The autolysate from the culture of tubercle bacilli *E* (now designated autolysate *E*) was inoculated into Löwenstein medium, together with a suspension of living tubercle bacilli (strain *Y*). One week later no colonies had developed while simultaneous control subcultures of strain *Y* yielded abundant growth of tubercle bacilli. Fifteen days after inoculation the negative subcultures developed a few colonies of tubercle bacilli while simultaneous control subcultures of strain *Y* showed an abundant growth of tubercle bacilli.

Autolysate *E* was inoculated further into Löwenstein medium together with the strain of tubercle bacilli (*E*) from which the autolysate had originally been derived. A week later no colonies had developed, while simultaneous control subcultures of strain *E* were positive. A month after inoculation, subcultures which also contained autolysate *E* were still sterile, while control subcultures showed abundant growth of tubercle bacilli.

Three tubes of Löwenstein medium were inoculated with autolysate *E* for the purpose of finding out whether it still contained viable tubercle bacilli. No growth was observed in the tubes after a month. In order to find out if inapparent growth had occurred in these tubes, material was taken from them and inoculated along with living tubercle bacilli (strain *Y*) into Löwenstein medium.

In order to determine whether autolysate *E* was still active, it was inoculated into three tubes of Löwenstein medium, together with living tubercle bacilli from strain *E*. No growth was observed after a month while the control tubes showed the usual growth of tubercle bacilli after inoculation.

Autolysates *E* and *A*, respectively, were incubated with nonpathogenic acid-fast bacilli (*b.* Orange smooth, Communicable Disease Center, Atlanta, Georgia) in Löwenstein medium. No inhibition of growth was observed and colonies of tubercle bacilli appeared within forty-eight hours, as was the case also in the control tubes.

In summary: A factor inhibitory for tubercle bacilli of human origin has been obtained irregularly from autolysates of cultures of certain strains of tubercle bacilli. The inhibitory factor survived filtration and is believed to represent a bacteriophage.

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A COMPARATIVE STUDY OF THREE EGG YOLK MEDIA IN THE ISOLATION OF M. TUBERCULOSIS

The three egg-yolk media most commonly used in diagnostic laboratories are American Trudeau Society (ATS),[1] Löwenstein–Jensen,[2] and Petragnani.[3] As there were no definite data concerning their comparative growth-enhancing properties for the tubercle bacillus or their inhibitory properties for contaminants, the present study was undertaken.

Under routine conditions, it is extremely difficult to distribute the inoculum evenly among a series of tubes. The importance of this problem is even more apparent when only a few organisms are present in the original sputum specimen. Therefore, large series of cultures are necessary to be of statistical significance when such a study is made.

In the present survey, 1,007 sputum specimens positive for *M. tuberculosis* were cultured with a total of 3,021 cultures. One hundred eighty-three specimens, with a total of 549 cultures, were cultured on ATS medium alone. Eight hundred twenty-four specimens, with a total of 2,472 cultures, were inoculated into all three media.

The specimens were seventy-two-hour collections of sputum homogenized by adding an equal amount of 23 per cent trisodium phosphate and shaking the mixture for five minutes in a paint conditioner machine. The mixture was incubated at 37°C. for sixteen hours and the sediment was neutralized to pH 6.8. The sediment was then divided into three equal parts for inoculation of three tubes of media.

The study of ATS medium alone served not only as a control but also helped to establish whether or not the number of cultures positive for *M. tuberculosis* was increased by using more than one tube of medium for each specimen.

It will be noted in table 1 that among 549 ATS cultures, 375 (68.3 per cent) were positive for *M. tuberculosis*, 141 (25.7 per cent) showed no growth, and 33 (6.0 per cent) were contaminated.

It is evident from table 2 that, among 183 specimens inoculated into three tubes of ATS each, 73 showed growth in all three tubes. Thus, growth of tubercle bacilli would have been detected in 39.9 per cent of cases independent of the number of tubes of media used. On the other hand, in 46 cases (25.1 per cent), one tube of three was either contaminated or did not show growth. In these circumstances, tubercle bacilli might not have been detected if only one tube of medium were used. Furthermore, in 64 cases (35.0 per cent), two of three showed no growth of tubercle bacilli, indicating that in such cases growth could have been missed were only two tubes of media used.

In tables 3 and 4 are shown the comparative number of tubes with growth of *M. tuberculosis*, those with no growth, and those contaminated, when 824 specimens were inoculated into all three media. The highest percentage of cultures showing growth of *M. tuberculosis* (72.6 per cent) was obtained in the ATS medium, with Löwenstein–Jensen second (62.9 per cent), and Petragnani third (54.0 per cent). Tubercle bacilli failed to grow in 41.5 per cent of the tubes of Petragnani, 29.6 per cent of Löwenstein–Jensen, and in 20.2 per cent of ATS media. Contamination was slightly less in Petragnani (4.5 per cent) than in ATS (7.2 per cent) and Löwenstein–Jensen (7.5 per cent).

[1] Steenken, W., Jr.: Medium recommended on evaluation of laboratory procedures of the American Trudeau Society for the culture of tubercle bacilli.

[2] Löwenstein medium—Jensen modification 273-TS, B. B. L. Products for the Bacteriological Laboratory, ed. 2, 1950, p. 291.

[3] Petragnani medium B 1010T. Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures, ed. 9, 1953, p. 339.

TABLE 1

RESULT OF CULTURING 183 SPUTUM SPECIMENS POSITIVE FOR M. TUBERCULOSIS ON AMERICAN TRUDEAU SOCIETY MEDIUM

Number of Specimens	Number of Cultures	Positive Cultures	Per Cent	Negative Cultures	Per Cent	Contaminated Cultures	Per Cent
183	549	375	(68.3)	141	(25.7)	33	(6.0)

TABLE 2

THE NUMBER OF SPECIMENS SHOWING GROWTH OF M. TUBERCULOSIS IN ONE, TWO, OR THREE TUBES WHEN 183 SPECIMENS WERE EACH INOCULATED INTO THREE TUBES OF AMERICAN TRUDEAU SOCIETY MEDIUM

	Number of Specimens	Per Cent
Three tubes of three showed growth of *M. tuberculosis*	73	39.9
Two tubes of three showed growth of *M. tuberculosis*	46	25.1
One tube of three showed growth of *M. tuberculosis*	64	35.0

TABLE 3

RESULT OF CULTURING 824 SPUTUM SPECIMENS POSITIVE FOR M. TUBERCULOSIS ON AMERICAN TRUDEAU SOCIETY, LÖWENSTEIN-JENSEN, AND PETRAGNANI MEDIA

Number of Specimens	Number of Cultures	Positive Cultures	Per Cent	Negative Cultures	Per Cent	Contaminated Cultures	Per Cent
824	2,472	1,560	(63.0)	753	(30.5)	159	(6.4)

TABLE 4

A COMPARISON OF THE GROWTH OF M. TUBERCULOSIS ON THREE EGG-YOLK MEDIA INOCULATED WITH 824 POSITIVE SPECIMENS OF BACILLUS-CONTAINING SPUTUM

Medium	Positive Cultures		Negative Cultures		Contaminated Cultures	
	Number of Tubes	Per Cent	Number of Tubes	Per Cent	Number of Tubes	Per Cent
American Trudeau Society	597	72.6	167	20.2	60	7.2
Löwenstein-Jensen	518	62.9	244	29.6	62	7.5
Petragnani	445	54.0	342	41.5	37	4.5

TABLE 5

THE NUMBER OF SPECIMENS IN WHICH DIFFERENT RESULTS WERE OBTAINED WITH AMERICAN TRUDEAU SOCIETY, LÖWENSTEIN-JENSEN, AND PETRAGNANI MEDIA

American Trudeau Society + * Löwenstein-Jensen —		American Trudeau Society — Löwenstein-Jensen +		American Trudeau Society + Petragnani —		American Trudeau Society — Petragnani +	
Number of Specimens	Per Cent	Number of Specimens	Per cent	Number of Specimens	Per Cent	Number of Specimens	Per Cent
195	23.7	79	9.6	249	30.2	51	6.2

* + indicates growth of *M. tuberculosis*.
— indicates no growth of *M. tuberculosis*.

It is obvious that, under the conditions of this study, the most sensitive medium for growth of *M. tuberculosis* was the American Trudeau Society medium. This fact can be noted in table 4 and also may be seen by comparing the total number of positive cultures on ATS alone (table 1) with the total positive cultures of all three media (table 3). The same conclusion can be drawn from table 5, which represents the discrepancies found when 824 specimens were cultured on the three media. It will be noted that, when such differences existed, the ATS medium produced a far greater number of cultures positive for *M. tuberculosis*.

The most inhibitory medium, not only for contamination but also for the tubercle bacillus, proved to be Petragnani as evidenced by the data in table 4.

In summary: Under the routine laboratory conditions of collection, processing, and culturing of specimens, there was no advantage in using media other than the American Trudeau Society medium provided that three tubes of this medium were used for each specimen.

LABORATORY SERVICE
VETERANS ADMINISTRATION HOSPITAL
BRONX 68, NEW YORK

MICHAEL KENNEY[1]
PEGGY M. JOHNSON
MILTON GOLDMAN

June 15, 1955

[1] Present address: Laboratory Service, Veterans Administration Hospital, New York 10, New York.

Correspondence

VETERANS ADMINISTRATION–NATIONAL TUBERCULOSIS ASSOCIATION COOPERATIVE STUDY OF MYCOBACTERIA

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

The unidentified acid-fast organisms which are distinct in colony, cell, and virulence characteristics from the usual tubercle bacilli have always been a problem to the tuberculosis bacteriologist. The increasingly frequent finding that some of these strains are the only evident agents of associated pulmonary or other disease has, for many reasons, made imperative the comparison of as much pertinent clinical and bacteriological data as possible.

The Veterans Administration Subcommittee on Atypical Acid-fast Organisms (Ernest H. Runyon, William H. Feldman, Wendell H. Hall, and David T. Smith) drew up a statement and questionnaire which were printed by the American Trudeau Society and sent from Dr. Esmond Long's office to selected tuberculosis hospitals and from the Veterans Administration central office to other Veterans Administration Hospitals. The purpose of this was to invite participation in a survey of hospital experiences with unidentified mycobacteria and the establishment of a culture collection of these at Sunmount Hospital. The following preliminary statement is a summary of current indications resulting from this survey to date.

1. Something as to the incidence of unidentified strains of mycobacteria associated with human disease may be seen from the following data:

Number of hospitals to which questionnaires were sent	190
Number of hospitals reporting significant[1] strains	33
Number of hospitals reporting other strains	41
Number of hospitals reporting no significant strains encountered	24
Number of hospitals reporting, total	98

* * * * * *

Number of patients having infections with significant strains reported by the 33 hospitals	156
Number of patients whose cultures are available at the Sunmount Collection, more than	200

2. The occurrence of unidentified mycobacteria which by evidence of their source are possibly pathogenic for man is widespread. Some laboratories handling large numbers of cultures, however, have not recognized or recorded the occurrence of these strains.

3. Significant[1] strains are of more than one kind.

4. Two types, each evidently independently pathogenic in lung tissue, have been met with repeatedly and at widely separated areas in the United States. Other types are reported to have been involved in superficial lesions or in pulmonary disease in which there is concomitant pneumoconiosis.

5. The two types of presumptive pulmonary pathogens are:

(*a*) Photochromogenic strains. These form a lemon-yellow pigment during growth if exposed to light. Colonies are usually smooth.

[1] By "significant" is meant isolated either from lung tissue or repeatedly from the same patient and in the absence of typical tubercle bacilli.

(*b*) Non-photochromic strains. These are white, beige, or buff to yellowish; the pigmentation, if any, is not light-conditioned. Colonies are smooth.

6. The distribution of these two types in the United States is yet to be determined. Some laboratories have reported rather large numbers of only one of these types; other laboratories have encountered both.

7. Relation of chemotherapy to the occurrence of these forms is not evident in the data at hand. A large proportion was isolated prior to drug treatment.

8. Recognition of these "atypical" pathogens may be by one or more of the following characteristics:

(*a*) *Colonies* are distinct in color, shape, and texture. Most are somewhat pigmented and smooth; however, *some are non-pigmented*, and a few are rough.

(*b*) *Cord* formation is absent or less pronounced than for virulent *M. tuberculosis*. Dispersion in salt solution is easier.

(*c*) *Cells* are usually larger or smaller or otherwise of different shape than typical tubercle bacilli.

(*d*) *PAS resistance* is characteristic of a large number of these strains prior to chemotherapy.

9. Animal tests for indication of virulence for man may be unreliable. Most strains are reported nonvirulent for guinea pigs and rabbits. Many have been found to have some pathogenicity for mice and/or hamsters.

10. The most thorough search should be made to determine if any other source of disease may be present, such as tubercle bacilli, a fungus, inhaled or aspirated foreign material in the lung. A most important test is to inoculate guinea pigs with original *specimens* and, if lesions result, to culture material from them.

11. In many cases included in the figures of the first paragraph it is clear that no such thorough search as described above in item 10 was made. However, in other cases this search was made. The similarity of the photochromogenic strains in every case from widely separated patients lends support to the probability that we are dealing here with one pathogen. The same may be said of the non-photochromic strains, although they are less uniform, some being somewhat pigmented and others not so.

12. Negative as well as positive tuberculin reactions have been reported for experimental animals and for human patients infected with these strains.

13. Lung tissue of individual patients having one of these mycobacterioses may have some histopathologic characteristics slightly atypical of tuberculosis, but as a whole the variations observed are well within the limits of the manifestations of this disease.

14. Deeply chromogenic yellow-orange strains which are pigmented even in complete darkness (skotochromogenic) have been reported to occur very commonly. They may evidently be found occasionally in the normal throat or gastric content. When isolated from a patient having pulmonary disease, with few exceptions these skotochromogens have been found to be accompanied by another well-recognized disease agent: true tubercle bacilli or foreign material such as silica. They have not been found to be pathogenic for any laboratory animal.

Continued collaboration in this cooperative study is highly desirable. Accumulation of information in regard to the following matters will aid in the understanding of these mycobacterioses:

Response to therapy
Tuberculin reactions
Careful follow-up of every case
Search for atypical organisms directly in diseased human tissue

In summary: This is a progress report of an investigation sponsored jointly by the Veterans Administration and the National Tuberculosis Association. A widespread occurrence of two kinds of mycobacteria distinct from human tubercle bacilli but evidently associated with human pulmonary disease is indicated.

June 24, 1955

VETERANS ADMINISTRATION HOSPITAL
SUNMOUNT, NEW YORK

REPORT OF THE PANEL ON ATYPICAL ACID-FAST BACILLI

ANNUAL MEETING OF THE NATIONAL TUBERCULOSIS ASSOCIATION, MILWAUKEE, MAY 25, 1955

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

In view of the somewhat nebulous impressions which resulted from the discussion in Milwaukee, members of the panel on "Atypical Acid-fast Bacilli" would like to make the following statement:

Increasing attention is being paid to the motley strains of acid-fast organisms other than *M. tuberculosis* which appear sporadically in every tuberculosis laboratory. It is increasingly evident, on one hand, that a certain few of these strains may be responsible for human pulmonary disease. On the other hand, the innocuous nature of the most commonly occurring chromogenic strains and certain non-chromogens occurring with tubercle bacilli remains well established. True tubercle bacilli themselves are subject to variation. Those with atypical colonies can be recognized by subculture. Most such variants quickly revert to typical rough, buff colonies. Other variants may have altered metabolism, drug susceptibility, or virulence. These strains however, are readily recognized as tubercle bacilli, for rarely, if ever, are they atypical simultaneously in multiple characteristics such as cell and colony shape, cell arrangement, consistency, and pigmentation. The "atypical" strains, on the contrary, regularly differ in most or all of these respects as well as in virulence from typical bacilli and hence are probably distinct taxonomically.

While some of these acid-fast organisms in common with tubercle bacilli may sometimes be observed to form short branching filaments, they are regularly bacilliform on the usual media and in animal tissues and are strongly acid-fast. In these respects they differ from the related genus *Nocardia*.

The strains of unclassified mycobacteria whose relationship to human pulmonary disease is at least reasonably probable have been shown by the "Cooperative Study of Mycobacteria" (*see* preceding letter) to be of at least two kinds: the photochromogenic and the non-photochromogenic. The former is the better defined. These strains become brilliant yellow if exposed to bright light, e.g., for one hour, and then are allowed to continue growth in the incubator. If grown in complete darkness they form no pigment.

A simple test to determine whether a given acid-fast organism is significant in human disease unfortunately is lacking. Indeed it must be pointed out that the mere occurrence of the same type repeatedly from the same patient and in the absence of *M. tuberculosis* is no demonstration of pathogenicity. Nor is the production of lesions in mice or hamsters any proof of their potential pathogenicity for men. Each occurrence of an atypical form must be considered in the light of the patient's status and history. Certainly, the ruling out of concurrent infection by recognized pathogens is of primary importance. For tubercle bacilli, the use of guinea pigs inoculated directly with specimens from the patient and culture of material from any resulting lesions are clearly indicated.

The new 1955 edition of *Diagnostic Standards* carries the following advice regarding "Unclassified (Atypical) Mycobacteria," ". . . it is an error to discard acid-fast organisms

isolated from pathological material if they fail to fit the usual cultural or virulence pattern of *M. tuberculosis*. Wherever possible such organisms which are repeatedly isolated from pathological material or specimens obtained from patients should be sent to laboratories prepared to make special studies or should be examined carefully by cultural and animal inoculation techniques employing mice, as well as guinea pigs, for determination of virulence." The members of the Panel suggest that, in addition to the animals mentioned, Syrian golden hamsters be inoculated intraperitoneally.

In order to further cooperative investigation in this important field, hospitals encountering strains of *Mycobacterium* distinct from *M. tuberculosis hominis* or *bovis* and evidently significant in human disease are urged to report or continue to report their findings to:

Dr. Ernest H. Runyon
Veterans Administration Hospital
Sunmount, New York

Report forms for this purpose may be obtained from him, but are not required. Cultures accompanied by the pertinent information may be sent to the same address. The culture collection makes possible comparison of scattered and divergently reported strains and is a source available to any investigator.

Respectfully submitted by members of the panel.
August 10, 1955

Mary Clark
Wendell H. Hall
Ann Pollak
Ernest H. Runyon
Lawrence E. Wood
Eugene Woodruff
Diran Yegian
William H. Feldman, *Moderator*

INFECTION PRODUCED BY BCG

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

French and other European readers have certainly taken great interest in the report by Masashi Tamura *et al.*, published in the American Review of Tuberculosis (1955, *71*, 465) on an epidemic of cutaneous and lymphatic tuberculosis following massive vaccination of children with anti-typhoid-paratyphoid vaccine. But they must have been greatly surprised to see that the authors had not heard of this problem and of its unhappy issue, since the inoculation of tuberculosis through injections is a well-known topic in Europe. The question was studied and reports were published in Great Britain, as well as in Germany, Czechoslovakia, and France, of cases occurring after injections of penicillin, of convalescent serum, of quinine, of vitamins, of various vaccines, even of saline.

The cause of these infections has now been clearly established and lies in the inadequate disinfection of the syringe used for injections performed on several patients. This is so well known that, from the start, French doctors and medical students called it syringe pathology. All is not quite clear in every particular case, but the general process has been soundly established. Some accidents, which had to be brought before French courts, occurred, as they did in Japan, after massive vaccinations of children at school. The French Academy of Medicine thought it wise to advise special methods for the disinfection of syringes so as to avoid similar regrettable accidents.

With Professor Maurice Lamy and other co-workers, I published some time ago an extensive review entitled "Primary Tuberculosis by Injections in Children" (Ann. méd., 1951, *52*, 433). In this paper (63 pages) I reported 58 cases that I had personally studied and gave 38 references (French, German, and English).

Later, I was appointed as expert by the French Court, together with my colleague Etienne Bernard, and I studied 65 cases chosen among cases of localized tuberculosis following massive vaccinations. I did not publish these reports since they are known to everybody in Europe.

If I mention this, it is of course not to claim vain priority in the knowledge of this mechanism—I feel such quarrels futile, especially in this case—but because once more I feel sorry that authors in many countries keep to the literature in one language. The classical works in medicine and biology written in French or German remain unknown to authors using another language. This is misleading and liable to make them discover facts already observed by others. Isn't it a pity?

Clinique Médicale des Enfants
149 Rue de Sèvres
Paris—XV
France

Robert Debré

September 10, 1955

Books

THE HISTORY AND CONQUEST OF THE COMMON DISEASES. Edited by Walter R. Bett, Pp. 334, University of Oklahoma Press, Norman, Oklahoma, 1954, Price, $4.00.

As popular entertainment for the layman, medicine and things medical have become a fad. Witness only the great number of popular radio and television shows dealing with medical subjects, and the number of related popular short stories and novels on the best seller lists. This volume, so excellently edited by Dr. Walter R. Bett, Librarian to the Royal Society of Medicine, while "intended mainly for patients" will find its usefulness and value mainly appreciated by the physician and student of medicine. It makes for good reading and entertainment (a tribute to good editing, for each chapter is by a different writer). The presentations are accurate, well documented, and quite technical and for these reasons probably "over the head" of the lay public for which it is intended. On the other hand, for the physician and student some of the presentations are quite abbreviated and thereby less attractive. The volume does not pretend, however, to be a medical history in any sense of the word. Diverse subjects, all common diseases in themselves, are considered by one or several authorities in the field from an historical standpoint. The focal point of each chapter is the disease process, and the story is that of the disease. Thus, this book does not fall into the trap, in which so many such books get enmeshed, of becoming a compilation of hundreds of paragraphs, each summing up the life and accomplishments of one great physician. Such diseases as pneumonia, rickets, epilepsy, and arthritis are discussed. A particularly delightful chapter is one on gallstones. The book is weak in spots, for some chapters attempt to cover too much and end up by falling far short of the intended goal. Such is true of the chapter on diseases of the endocrine glands. Certainly the exciting story of Addison's disease (in this reviewer's opinion) could be a chapter in itself.

The references given enhance the value of this book greatly. These alone would make the book eminently worth while and valuable to the medical teacher who makes use of the "Oslerian methods." In this situation it is a very handy reference book.

JOHN COLLINS HARVEY
Baltimore, Maryland

PULMONARY DISEASES. Edited by Roscoe L. Pullen. Pp. 669, with 195 illustrations, Lea & Febiger, Philadelphia, 1955, Price, $15.00.

This publication prepared by twenty contributing authors, including the editor, Roscoe L. Pullen, is a significant contribution to the dissemination of medical knowledge of the pulmonary manifestations of disease, which now constitute such an important proportion of the clinical problems faced by practicing physicians.

The introductory chapters, in which the normal and abnormal anatomic and physiologic aspects of the ventilatory, gaseous exchange, and transport systems concerned with respiration are discussed, are succinct and wholly adequate for the pragmatic application of the material presented in subsequent sections.

The syndromes and specific disease entities discussed by the various contributors are arranged in logical sequence; beginning with infections of the upper respiratory passages, through bronchitis, the viral, bacterial, and mycotic pneumonias, to the neoplastic diseases, structural changes, developmental abnormalities and manifestations of trauma. The sections on bronchiogenic neoplasm, tuberculosis, and the pulmonary mycoses are par-

ticularly well done and timely. The other principal manifestations of pulmonary disease are clearly presented.

The illustrations have been chosen carefully and are reproduced with clarity. References given at the end of each section lend authority to the presentations and offer excellent suggestions to one who wishes to pursue any subject further.

The editor of this book is to be complimented on his choice of contributors and his skillful arrangement of the material. This publication should fill a most important place in stimulating interest and enhancing medical ability in the diagnosis and management of these increasingly significant clinical entities.

WINTHROP N. DAVEY
Ann Arbor, Michigan

THE HEALTH VISITOR AND TUBERCULOSIS. By Sheena H. Buchanan. Pp. 146, The National Association for the Prevention of Tuberculosis, London, England, 1955. Price, $2.00.

This book is directed towards developing a greater understanding of the role of the health visitor in the prevention of tuberculosis and the care of the patient and his family during the total treatment process. In its ten chapters, the incidence of tuberculosis, method of contact investigation, treatment techniques, and health legislation in Great Britain pertaining to available services are presented. Within this framework, the teaching and preventive aspects of the health visitor's service is described with some case illustrations. A plea for greater cooperation among medical and extra-medical professions is made. To readers in the United States, where the treatment function of the social worker and the teaching function of the public health nurse are more clearly defined, the differentiation of professional approach and areas of responsibility outlined in this book would need further clarification. Miss Buchanan's book should undoubtedly be of interest to all professional groups in England concerned with improving the care of the patient and his family and for more effective use of existing extra-medical services.

MARGARET A. DENNIS
New York, New York

BOOKS RECEIVED

WHYS AND WHEREFORES IN TUBERCULOSIS. By George Day. Pp. 44, The National Association for the Prevention of Tuberculosis, London, England, 1955, Price 3s. 6d.

TUBERCULOSIS AND THE INDIVIDUAL. By J. S. Campbell. Pp. 92, The National Association for the Prevention of Tuberculosis, London, England, 1955, Price 5s. 0d.

LA TUBERCULOSIS TRÁQUEOBRONQUIAL. By Juan Carlos Dighiero. Pp. 129, Instituto de Tisiologia, Montevideo, Uruguay, 1954.

RÖNTGENSCHICHTVERFAHREN. By R. Griesbach and F. Kemper. Pp. 143, Georg Thieme Verlag, Stuttgart, Germany, 1955, Price $9.30.

TRANSACTIONS OF THE 14TH CONFERENCE ON THE CHEMOTHERAPY OF TUBERCULOSIS. Veterans Administration, Army, and Navy. Pp. 556. Atlanta, Georgia, February 7–10, 1955.

CLINICAL ROENTGENOLOGY. Vol. III. By A. A. de Lorimier, H. G. Moehring, and J. R. Hannan. Pp. 508, Charles C Thomas, Springfield, Illinois, 1955, Price $20.50.

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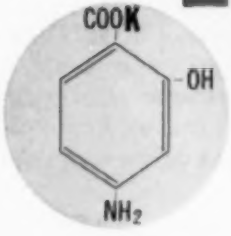

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1. *Molthan, L., Cohen, B. V., and Zarafonetis, C. J. D.: Clinical use of potassium para-aminosalicylate (KPAS). Am. Rev. of Tuberc. and Pulmon. Dis. 71:220 (Feb.) 1955.*

2. *Cohen, B. V., Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations). Read before the American Trudeau Society, Annual Meeting, Milwaukee, Wisconsin, May 23-27, 1955.*

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1. Recommended Dietary Allowances, Washington, D. C., National Academy of Sciences—National Research Council, Publication 302, 1953.
2. Co Tui: Review: The Fundamentals of Clinical Proteinology, J. Clin. Nutrition *1*:232 (Mar.-Apr.) 1953.
3. Keys, A.; Brozek, J.; Henschel, A.; Mickelsen, O., and Taylor, H. L.: The Biology of Human Starvation, Minneapolis, Univ. of Minnesota Press, 1950.
4. Burger, G. C. E.; Drummond, J. C., and Sandstead, H. R.: Malnutrition and Starvation in Western Netherlands, The Hague General State Printing Office, 1948, Part II, p. 91.
5. Co Tui; Kuo, N. H.; Chuachiaco, M., and Mulholland, J. H.: The Protein Depletion (Hypoproteinia) Syndrome and Its Response to Hyper-Proteinization, Anesth. & Analg. *28*:1 (Jan.-Feb.) 1949.

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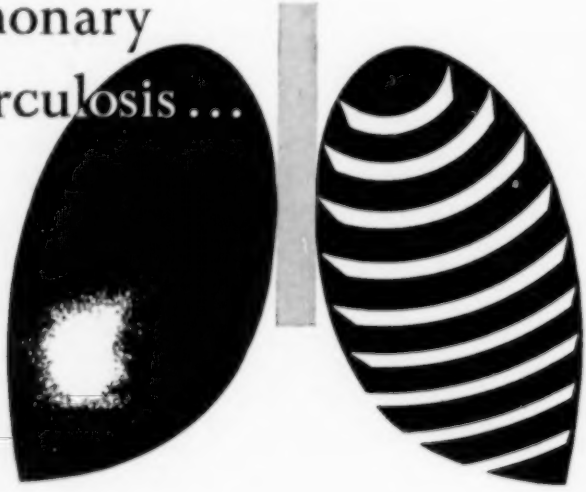

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1. Dobkin et al.: Anaesthesia 10:328 (Oct.) 1955.

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